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Serial Number: 09/167,088 Results Format Preferred (circle): PAPER DISK E-MAIL

Title of Invention: Method for Measuring In Vivo Cytokine Production

Inventors (please provide full names): Fred D. Finkelman, Suzanne C. Morris

Earliest Priority Date: 10-6-97

Keywords (include any known synonyms registry numbers, explanation of initialisms):
Target analyte = macromolecule = protein = cytokine
Cytokine = interleukins (1-18)
interferons (alpha, beta, gamma
chemokines, lymphotoxins, lymphokines
growth factors, colony stimulating factors
tumor necrosis factors (alpha)
TGF-beta, GM-CSF, NGF, and EGF
Targeting moiety = antibodies, soluble receptors,
paratopic molecules, recombinant mol...
→ binding site for cytokines
Capture moiety = polyclonal antibody (many epitopes)
Search Topic: Targeting moiety = detectable label = radioisotopes, enzymatic, fluorescent, alkaline

Search Topic:
Please write detailed statement of the search topic, and the concept of the invention. Describe as specifically as possible the subject matter to be searched. Define any terms that may have a special meaning. Give examples of relevant citations, authors, etc., if known. You may include a copy of the abstract and the broadcast or most relevant claim(s).

See claim 1 and incorporate keywords
* A method and kit for measuring cytokine producti...
inside the human/animal body.

Keywords continued
Label = small molecule hapten = biotin
Enzyme conjugated binding partner = streptavidin /
anti-biotin, anti-hapten, ...

854
5.28

435
691.11

Point of Contact:
Mary Hale
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CM1 12D16 Tel: 308-4258

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167088

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          1997 INTERLEUKIN?/CN
           600 INTERFERON?/CN
           114 CHEMOKINE?/CN
            46 LYMPHOTOXIN?/CN
           238 LYMPHOKINE?/CN
             0 GROWTH FACTOR/CN
             0 COLONY STIMULATING FACTOR/CN
           587 TUMOR NECROSIS FACTOR?/CN
L1        3548 (INTERLEUKIN? OR INTERFERON? OR CHEMOKINE? OR LYMPHOTOXIN? OR
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               TUMOR NECROSIS FACTOR?)/CN

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igg)/cn

             1 STREPTAVIDIN/CN
             0 ANI BIOTIN/CN
             0 "ANTI-BIOTIN"/CN
             0 "ANTI-HAPTEN"/CN
             0 ANI IGG/CN
L2           1 (STREPTAVIDIN OR ANI BIOTIN OR "ANTI-BIOTIN" OR "ANTI-HAPTEN"
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factor? or l1 or cytokine) and (target analyte or macromolecule or protein)

L3       100758 FILE MEDLINE
L4        81387 FILE CAPLUS
L5        77134 FILE BIOSIS
L6        81051 FILE EMBASE
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L12      18647 FILE EMBASE
L13       1180 FILE WPIDS

TOTAL FOR ALL FILES
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radioisotope or enzymatic or fluorescen? or affinity)

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L16       4269 FILE CAPLUS
L17       3565 FILE BIOSIS
L18       5445 FILE EMBASE
L19        285 FILE WPIDS

TOTAL FOR ALL FILES
L20      21274 L14 AND (POLYCLONAL ANTIBOD? OR EPITOPE OR DETECTABLE LABEL OR
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=> s (streptavidin or anti biotin or "anti-biotin" or "anti-hapten" or ani
igg or l2) and l20

L21          24 FILE MEDLINE
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L23          13 FILE BIOSIS
L24          24 FILE EMBASE
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L25           3 FILE WPIDS

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               OR ANI IGG OR L2) AND L20

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PROCESSING COMPLETED FOR L26
L27          68 DUP REM L26 (34 DUPLICATES REMOVED)

=> d cbib abs 1-68;s finkelman f?/au,in;s morris s?/au,in
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L27 ANSWER 1 OF 68 CAPLUS COPYRIGHT 1999 ACS
1999:222965 Catalytic **antibodies** and a method of producing same. Koentgen, Frank; Suess, Gabriele Maria; Tarlinton, David Mathew; Treutlein, Herbert Rudolf (Amrad Operations Pty. Ltd., Australia). PCT Int. Appl. WO 9915563 A1 19990401, 101 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 98-AU783 19980918. PRIORITY: AU 97-9306 19970919.

AB The present invention relates generally to a **growth factor** precursor and its use to select prodn. of antigen specific catalytic **antibodies**. Such catalytic **antibodies** are produced following B cell activation and proliferation induced by catalytic cleavage products of a target antigen portion of the **growth factor** precursor of the present invention. A particularly useful form of the **growth factor** precursor is as a nucleic acid vaccine. The nucleic acid vaccine of the present invention preferably further comprises a mol. adjuvant. Another aspect of the present invention comprises a **growth factor** precursor in multimeric form. The **growth factor** precursor of the present invention is useful for generating catalytic **antibodies** for both therapeutic, diagnostic and industrial purposes, esp. for treating rheumatoid arthritis, AIDS and Alzhemer's disease and others. Thus, pÄSK75 encoding ompA signal sequence and LHL was constructed and expressed in Escherichia coli, and LHL was purified over a human IgG **affinity** column. Similarly, LHL.seq contg. N-terminal FLAG **epitope** (DYKDDDDK) and C-terminal strep-tag (AWRHPQFGG) was generated, while the FLAG **epitope** was added to facilitate the secretion of LHL.seq and strep-tag was added for purifn. by **streptavidin** column. TLHL comprising FLAG-kappa-linker-tobacco etch virus protease (TEV)-LHL-strep-tag was also generated and CATAB-TEV was assembled from TLHL and kappa by PCR. B cell proliferation and activation, B7-1 expression, MHC class II induction, detection of CATAB-specific catalytic **antibodies** in serum, OMP-induced multimerization, design of novel multimeric mitogen, etc. were tested with the prepd.

L27 ANSWER 2 OF 68 CAPLUS COPYRIGHT 1999 ACS
1999:100795 Document No. 130:135882 **Affinity** ultrafiltration assay for determining transferase activity. Huang, Eric Z. (USA). U.S. US 5869275 A 19990209, 11 pp. (English). CODEN: USXXAM. APPLICATION: US 98-118900 19980720.

AB The present invention relates to a method for assaying transferase activity upon incorporation of an **affinity** ultrafiltration process as a sepn. means, which method comprises: (1) reacting the transferase to be assayed with a labeled substrate and an unlabeled substrate to yield a product having a label moiety from the labeled substrate and a **binding site** moiety either from the unlabeled substrate or existing as a specific structure of the product, (2) contacting the reaction mixt. with a sol. macroligand capable of forming a specific complex with the product via the **binding site** moiety, (3) subjecting the complex mixt. to ultrafiltration

which retains the complex and passes the unreacted labeled substrate, (4) washing the retentate, and (5) detg. the final retentate. Specific examples of transferases which can be assayed using the **affinity** ultrafiltration assay method include **protein** kinase, farnesyl transferase, thymidine kinase, chloramphenicol acetyltransferase and neomycin phosphotransferase. The present invention also relates to a kit embodying the inventive concept of the **affinity** ultrafiltration assay for transferase activity.

L27 ANSWER 3 OF 68 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
1999100103 EMBASE Non-invasive drug delivery to the human brain using endogenous blood- brain barrier transport systems. Pardridge W.M.. W.M. Pardridge, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90095-1682, United States. wpardrid@med1.medsch.ucla.edu. Pharmaceutical Science and Technology Today 2/2 (49-59) 1999. Refs: 50.
ISSN: 1461-5347. CODEN: PSTTF8.
Publisher Ident.: S 1461-5347(98)00117-5. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB Brain drug development is limited by the blood-brain barrier (BBB), which restricts the passage into the brain of >95% of all drug candidates intended for the CNS. The growth of future CNS drug development can be accelerated by fostering parallel growth in both CNS drug discovery and CNS drug delivery. One approach to solving the BBB problem is to target endogenous BBB transport systems, and to develop CNS drug delivery strategies that take advantage of these natural portals of entry into the brain.

L27 ANSWER 4 OF 68 CAPLUS COPYRIGHT 1999 ACS
1999:7860 Document No. 130:65250 **Chemokine** receptor-binding oligopeptides or nonpeptidyl agents for preventing HIV-1 infection of CD4+ cells. Allaway, Graham P.; Litwin, Virginia M.; Maddon, Paul J.; Olson, William C. (Progenics Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 9856421 A1 19981217, 85 pp. DESIGNATED STATES: W: AU, CA, JP, MX, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 98-US12331 19980612. PRIORITY: US 97-876078 19970613.
AB This invention provides methods for inhibiting HIV-1 infection of CD4+ cells which comprise contacting CD4+ cells with a non-**chemokine** agent capable of binding to a **chemokine** receptor in an amt. and under conditions such that fusion of HIV-1 to the CD4+ cells is inhibited, thereby inhibiting the HIV-1 infection. The non-**chemokine** agent is an **antibody** or its oligopeptide deriv., and a nonpeptidyl agent, which is not a known bicyclam or its known deriv. The non-**chemokine** agent polypeptide may be MIP-1.beta. sequence with deletion of first seven N-terminal amino acids or SDF-1 sequence with deletion of first six N-terminal amino acids. The **chemokine** receptor is CCR5 or CXCR4.

L27 ANSWER 5 OF 68 CAPLUS COPYRIGHT 1999 ACS
1998:761906 Document No. 130:11283 Production and purification of nucleic acid molecules using **affinity**-labeled primer-adapter molecules. Gruber, Christian E.; Jessee, Joel A. (Life Technologies, Inc., USA). PCT Int. Appl. WO 9851699 A1 19981119, 50 pp. DESIGNATED STATES: W: JP; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 98-US9586 19980512. PRIORITY:
US 97-46219 19970512.

AB The present invention is directed to methods for the prodn. and isolation of nucleic acid mols. In particular, the invention concerns isolation of mRNA mols. and the prodn. and isolation of nucleic acid mols. (e.g., cDNA mols. or libraries), which may be single- or double-stranded. Addnl., the invention concerns selection and isolation of particular nucleic acid mols. of interest from a sample which may contain a population of mols. Specifically, the invention concerns **affinity**-labeled primer-adapter mols. which allow improved isolation and prodn. of such nucleic acid mols., increasing both product recovery and speed of isolation. A mRNA or poly(A)+ RNA mols is mixed with a polymerase and/or reverse transcriptase and a primer-adapter nucleic acid mol., wherein the primer-adaptor comprises one or more ligand mols. and one or more cleavage sites (for endonuclease or restriction endonuclease). The primer-adaptor may be designed to hybridize to any portion of the template. Upon incubation under appropriate conditions, a first nucleic acid mol. (e.g., a single-stranded cDNA) complementary to all or a portion of the template is made, which contains the primer-adaptor and thereby facilitates isolation of the first nucleic acid mol. and/or any nucleic acid mol. hybridized to the first nucleic acid. Such isolation may be accomplished by ligand-hapten interactions, where the hapten is bound to a solid support. Multiple synthesis with primer-adaptor mols. may result in a synthesized nucleic acid mol. having more than one primer-adaptor. The present invention is particularly suited for the rapid prodn. and isolation of cDNA libraries from small amts. (as little of 5 ng) of input poly(A)+ RNA or mRNA in a high-throughput manner. About 3-4-fold greater yield of cDNA is achieved by the present invention than with the SUPERSCRIPT Plasmid System, with approx. equiv. transformation efficiencies and av. insert sizes.

L27 ANSWER 6 OF 68 CAPLUS COPYRIGHT 1999 ACS

1998:712383 Document No. 129:311692 Hybridization assay in which excess probe is destroyed enzymically and duplex is detected directly by **protein** binding. Harbron, Stuart (UK). PCT Int. Appl. WO 9846790 A1 19981022, 36 pp. DESIGNATED STATES: W: AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GE, GW, HU, ID, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 98-GB1057 19980409. PRIORITY: GB 97-7531 19970414.

AB A method is disclosed for detecting single-stranded target nucleic acid (2) which comprises the steps of forming a hybrid between said target nucleic acid and a nucleic acid probe (4), said nucleic acid probe labeled with an enzyme reagent (6) which hydrolyzes single-stranded nucleic acid but is substantially without effect on double-stranded nucleic acid, said hybrid formed under conditions of pH which are outside the activity range of said enzyme reagent, adjusting said pH to a value within the activity range of said enzyme reagent, allowing said enzyme reagent substantially to hydrolyze any single-stranded nucleic acid present, and detecting said hybrid.

L27 ANSWER 7 OF 68 CAPLUS COPYRIGHT 1999 ACS

1998:612214 Document No. 129:225709 Heterogeneous and homogeneous protease inhibitor assays using chemiluminescent 1,2-dioxetanes. Bronstein, Irena; Voyta, John; Palmer, Michelle; Tillotson, Bonnie (Tropix, Inc., USA). PCT Int. Appl. WO 9839471 A1 19980911, 42 pp. DESIGNATED STATES: W: AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 98-US3929 19980306.

PRIORITY:

US 97-38940 19970307.

AB Heterogenous and homogeneous assays are provided for the detection of protease inhibitory activity in a sample or target compd., taking advantage of the chemiluminescent characteristics of 1,2-dioxetanes. In the heterogeneous assay, a peptide bearing a cleavage site for the protease of interest is provided with a first member of a first ligand binding pair at one end, and a first member of a second ligand binding pair at the other end. The other member of the first ligand binding pair is attached to a surface, which binds the peptide, or protease substrate, to the surface. The peptide substrate is combined with the protease and target compd. or sample. Substrate cleavage, if not inhibited, is allowed to occur, and any unbound cleaved fragments are removed. An enzyme complexed with the second member of the second ligand binding pair is added, and allowed to bind to any of the (uncleaved) first member of the second ligand binding pair remaining. Unbound complex is removed, and a 1,2-dioxetane substrate for the enzyme is added. If any peptide substrate has not been cleaved, the dioxetane will chemiluminesce, indicating inhibitory activity. In a homogeneous assay, the same substrate bears at one end a **fluorescent** energy accepting moiety, and at the other end a 1,2-dioxetane or precursor. If the substrate is cleaved by the protease, the dioxetane and the **fluorescent** moiety are not in close phys. relationship, and no energy transfer occurs when the dioxetane is caused to decomp. If cleavage has not occurred, indicating inhibition, when the dioxetane is caused to decomp., energy is transferred to the fluorescing entity, which releases light of a wavelength recognizably distinct from that of the dioxetane. A heterogeneous assay for HIV-1 protease inhibitors was tested.

L27 ANSWER 8 OF 68 CAPLUS COPYRIGHT 1999 ACS

1998:398478 Document No. 129:51715 Chimeric integrin in methods and cell lines for identification of regulators of integrin activation and compositions identified thereby. Ginsberg, Mark H.; Fenczik, Csilla (Scripps Research Institute, USA). PCT Int. Appl. WO 9825144 A1 19980611, 20 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 97-US22263 19971202. PRIORITY: US 96-33248 19961206; US 97-948221 19971009.

AB A method for identifying regulators of integrin activation involve (a) establishing a selected cell line which contains a functional integrin and a chimeric polypeptide having a cytoplasmic domain of an integrin subunit fused to a polypeptide contg. extracellular and transmembrane domains that

are not functional integrin domains, so that the chimera can inhibit signaling activities of the functional integrin by interaction with integrin regulator mols. in the cytoplasm; (b) transfecting the cell line with a selected cDNA expression library; (c) expressing **proteins** of the cDNA expression library; and (d) identifying **proteins** which when overexpressed overcome the inhibition of signaling activities by the chimeric polypeptide, the **proteins** being regulators of integrin. Methods of designing drugs to modify integrin function and cell lines for screening regulators of integrin activation are also provided. CD98, an early T-cell activation antigen, was identified, through this method, as a regulator of integrin function. Further, it was detd. that the activity of this **protein** resides in the cytoplasmic tail of CD98, a small region susceptible to small mol. inhibition.

L27 ANSWER 9 OF 68 CAPLUS COPYRIGHT 1999 ACS
1998:478969 Document No. 129:133389 Identification of enantiomeric ligands. Schumacher, Antonius Nicolass Maria; Kim, Peter S. (Whitehead Institute for Biomedical Research, USA). U.S. US 5780221 A 19980714, 20 pp. Cont.-in-part of U.S. Ser. No. 482,309, abandoned. (English). CODEN: USXXAM. APPLICATION: US 96-627497 19960328. PRIORITY: US 95-433572 19950503; US 95-482309 19950607.

AB A method is disclosed for identifying **macromols**. (peptides, oligonucleotides, sugars and **macromol**. complexes, such as RNA-**protein** complexes, **protein**-lipid complexes), which are not of the natural handedness (not of the chirality as they occur in nature or as a wild type mol.) and which are ligands for other chiral **macromols**. A phage library was constructed in which random, 10-residue peptide sequences were expressed at the NH2-terminus of the pIII **protein** of the bacteriophage fd. The L-SH3 domain of chicken c-Src (prepd. by bacterial expression) was used to screen this phage display library for interacting peptide sequences. When the same phage display was screened with the D-SH3 domain (prepd. by chem. synthesis), a series of peptide sequences that showed no obvious sequence similarity to the L-SH3-binding sequences was isolated and grouped in three classes. These peptides all interact with the substrate-**binding site** of the SH3 domain. A D-peptide (Pep-D1) which is a mirror image of one of the phage-displayed peptides that bind to the D-SH3 domain was synthesized and its interaction with L-SH3 domain was examd. Heteronuclear magnetic resonance expts. were performed on 15N-labeled SH3 domain in the absence and presence of Pep-D1 to det. the **binding site** of the D-peptide in the SH3 domain.

L27 ANSWER 10 OF 68 CAPLUS COPYRIGHT 1999 ACS
1998:742323 Document No. 129:341453 Method for immunological-functional detection of biologically active insulin-like **growth factor** binding **proteins** or soluble insulin-like **growth factor**-receptors using an immunoassay. (Schuetzdeller, Angelika, Germany). Ger. Offen. DE 19719001 A1 19981112, 8 pp. (German). CODEN: GWXXBX. APPLICATION: DE 97-19719001 19970506.

AB The invention concerns a method for the detection of insulin-like **growth factor**-binding **proteins** (IGFBP) and sol. insulin-like **growth factor** receptors by an immunoassay that involves a specific **antibody** for the sepn. of the target compd. and the detection of the IGFBP with a labeled ligand. Ligands are insulin-like **growth factors** (IGF), their derivs., contg. at least 3 natural amino acid sequences, or their synthetic analogs. Any label for indirect or direct detection can be applied, e.g. labels for enzymic, radioactive, or **fluorescence** detection. Thus an enzyme immunoassay was developed for the detn. of IGFBP-3 with biotinylated IGF-1 and IGF-2 and **streptavidin**

-peroxidase detection. Blood serum, cell culture residue or cell ext. were the starting materials; the pH was set at 2.8 in order to remove bound IGFs. The mixt. was neutralized with a medium that contained biotinylated IGF-I, or IGF-II, or their mixt.; these were bound to all active IGFBP in the probe and displaced all of the non labeled IGF. The probe was transfered to the wells of a microtiter plate with immobilized **antibodies** to IGFBP-3. After incubation all IGFBP-3 were bound, the active an inactive ones. Following a washing step, avidin- or **streptavidin**-peroxidase was added, these reacted only with the IGF-I and IGF-II that was bound to active IGFBP-3; after adding the substrate, photometric measurement was carried out; the results were in correlation with the amt. of active IGFBP-3 in the probe.

L27 ANSWER 11 OF 68 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 98-361794 [31] WPIDS
CR 98-008044 [01]
AB US 5767287 A UPAB: 980805
Production of sulphoindocyanine dyes comprises reacting (a) 1st compound with an indolenine nucleus of formula (I); (b) second compound with an indolenine nucleus, which is the same or different from (a); and (c) reagent for linking (a) and (b) in a weakly basic solvent at reflux for at least one hour. X = CH3CCH3, O or S; Z = linkage group through which the indolenine nucleus is linked to A; and (A) = enzyme substrate or member of specific binding pair.

USE - The method is used in the manufacture of **fluorescent** sulphoindocyanine dyes, which can be used as labels for **antibodies**, DNA probes, biochemical analogues, lipids, drugs, **cytokines**, cells and polymers.

ADVANTAGE - The sulphoindocyanine dyes thus produced do not contain a reactive group that will covalently attach to a target molecule at a site such as an amine- or hydroxy-containing site, but are linked to an enzyme substrate or member of a specific binding pair, e.g. antigen/**antibody** systems, or hapten/antihapten systems, biotin/avidin, biotin/**streptavidin**, folic acid/folate-binding **proteins**, complementary probe nucleic acids, etc.
Dwg.0/1

L27 ANSWER 12 OF 68 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
1998138402 EMBASE T helper 1 (Th1) and Th2 characteristics start to develop during T cell priming and are associated with an immediate ability to induce immunoglobulin class switching. Toellner K.-M.; Luther S.A.; Sze D.M.-Y.; Choy R.K.-W.; Taylor D.R.; Maclennan I.C.M.; Acha-Orbea H..
K.-M.
Toellner, Department of Immunology, Univ. of Birmingham Medical School, Birmingham B15 2TT, United Kingdom. k.m.toellner@bham.ac.uk. Journal of Experimental Medicine 187/8 (1193-1204) 20 Apr 1998.
Refs: 70.
ISSN: 0022-1007. CODEN: JEMEAV. Pub. Country: United States. Language: English. Summary Language: English.
AB The respective production of specific immunoglobulin (Ig)G2a or IgG1 within 5 d of primary immunization with Swiss type mouse mammary tumor virus [MMTV(SW)] or haptenated **protein** provides a model for the development oft helper 1 (Th1) and Th2 responses. The **antibody**-producing cells arise from cognate T cell B cell interaction, revealed by the respective induction of C.gamma.2a and C.gamma.1 switch transcript production, on the third day after immunization. T cell proliferation and upregulation of mRNA for **interferon** .gamma. in response to

MMTV(SW) and **interleukin** 4 in response to haptenated **protein** also starts during this day. It follows that there is minimal delay in these responses between T cell priming and the onset of cognate interaction between T and B cells leading to class switching and exponential growth. The Th1 or Th2 profile is at least partially established at the time of the first cognate T cell interaction with B cells in the T zone. The addition of killed Bordetella pertussis to the hapten-**protein** induces nonhapten-specific IgG2a and IgG1 plasma cells, whereas the **anti-hapten** response continues to be IgG1 dominated. This indicates that a Th2 response to hapten-**protein** can proceed in a node where there is substantial Th1 activity.

L27 ANSWER 13 OF 68 MEDLINE DUPLICATE 1

1999162846 Document Number: 99162846. Clinical importance of c-Met **protein** expression in high grade astrocytic tumors. Hirose Y; Kojima M; Sagoh M; Hayashi T; Murakami H; Shimazaki K; Yoshida K; Kawase T. (Department of Neurosurgery, Ashikaga Red Cross Hospital, Tochigi.)NEUROLOGIA MEDICO-CHIRURGICA, (1998 Dec) 38 (12) 851-8; discussion 858-9.

Journal code: NYD. ISSN: 0470-8105. Pub. country: Japan. Language: English.

AB The clinical importance of the expression of c-Met **protein**, the receptor of hepatocyte **growth factor**/scatter factor, was evaluated in neuroepithelial tissue tumors. c-Met immunohistochemistry was performed using the **streptavidin**-biotin-peroxidase complex method with anti-c-Met **polyclonal antibody**. Specimens were classified as c-Met negative (< 30%) or c-Met positive (> or = 30%) according to the proportion of immunopositive cells under microscopic examination. All c-Met-positive cases occurred in high grade astrocytic tumors, not in other neuroepithelial tissue tumors. Most c-Met-positive astrocytic tumors were classified histologically as high grade tumors. Epidermal **growth factor**-receptor (EGFR) and MIB-1 immunohistochemistry were also performed for high grade astrocytic tumors.

Survival analysis was performed for patients with these tumors with variables including c-Met positivity, EGFR positivity, and MIB-1 labeling index. Positivity of c-Met was independent from EGFR positivity and MIB-1 labeling index, and the c-Met-positive group showed a significant shorter survival ($p < 0.05$). c-Met immunopositivity may be a parameter of biological aggressiveness in high grade astrocytic tumors. Examination of c-Met expression in astrocytic tumors provides significant clinical information, especially as a prognostic factor.

L27 ANSWER 14 OF 68 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2

1998:455781 Document No. 129:188149 Carrier-induced, hapten-specific suppression: a problem of antigen presentation?. Renjifo, Ximena; Wolf, Stanley; Pastoret, Paul-Pierre; Bazin, Herve; Urbain, Jacques; Leo, Oberdan; Moser, Muriel (Department de Biologie Moleculaire, Univ. Libre de Bruxelles, Rhode-Saint-Genese, Belg.). J. Immunol., 161(2), 702-706 (English) 1998. CODEN: JOIMA3. ISSN: 0022-1767. Publisher: American Association of Immunologists.

AB Prior immunity against a carrier **protein** has been shown to modulate the serol. response to injected haptens attached to the same carrier. In particular, a carrier/hapten-carrier immunization protocol induces marked suppression for IgG2a **anti-hapten** Ab prodn. but does not interfere with anti-carrier Ab responses. Although the phenomenon of epitopic suppression has been amply demonstrated, the mechanism underlying the suppression remains unknown. The selective

deficiency in IgG2a secretion suggests that IFN-.gamma.-producing Th1 cells are not properly activated. The authors and others have shown that the nature of the APCs present during the first encounter with the Ag influences the development of selected Th populations in vivo; dendritic cells (DCs) seem to be required for the induction of primary, Th1-type responses. Since carrier priming induces the clonal expansion of specific B cells that appear to efficiently capture the Ag, the authors hypothesized that the hapten-carrier conjugate may be presented by B cells in preimmunized animals. Therefore, the authors immunized mice to the conjugate by injecting syngeneic DCs pulsed in vitro with the Ag. The data show that an injection of DCs and IL-12 prevents epitopic suppression, suggesting that it may result from defective Ag presentation.

L27 ANSWER 15 OF 68 MEDLINE DUPLICATE 3

1998218938 Document Number: 98218938. Immunohistochemical examination of c-Met **protein** expression in astrocytic tumors. Hirose Y; Kojima M; Sagoh M; Murakami H; Yoshida K; Shimazaki K; Kawase T. (Department of Neurosurgery, Ashikaga Red Cross Hospital, Ashikaga-city, Tochigi, Japan.)ACTA NEUROPATHOLOGICA, (1998 Apr) 95 (4) 345-51. Journal code: 1CE. ISSN: 0001-6322. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Hepatocyte **growth factor**/scatter factor (HGF/SF), which has various physiological functions, and its receptor c-Met, the human c-met proto-oncogene product, are thought to be determinant in the pathological processes of various malignancies. To investigate the possible role of HGF/SF in the progression of development of astrocytic tumors, we examined the expression of c-Met in these tumors. Immunohistochemistry using the **streptavidin**-biotin peroxidase complex method and immunofluorescence double staining with anti-c-Met polyclonal and anti-glial fibrillary acidic **protein** monoclonal **antibodies** were performed. Positive c-Met expression was detected in 31 of the 42 astrocytic tumors and some of the control cases analyzed. c-Met-positive cells showed morphological characteristics of astrocytes. Especially in the cases of high-grade tumors, c-Met positivity was abundant in cells in both vascular-rich and peripheral regions of the tumors but not in the cells with distinctly malignant features. Immunofluorescence double staining revealed that the c-Met-positive cells were in part of astrocytic origin. We suggest that c-Met-positive cells are affected by some factors in the lesions where the pathological processes are in a state of development. Our studies indicated that c-Met expression might take part in glioma invasion but not in the development of malignancy.

L27 ANSWER 16 OF 68 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.

1998044868 EMBASE Basic fibroblast **growth factor** (FGF-2) in mouse tooth morphogenesis. Russo L.G.; Maharajan P.; Maharajan V.. Dr. V. Maharajan, CNR Institute of Cybernetics, 80072 Arco Felice, Naples, Italy. maha@maha.na.cnr.it. Growth Factors 15/2 (125-133) 1998. Refs: 28.
ISSN: 0897-7194. CODEN: GRFAEC. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB This study describes the spatio-temporal expression of basic Fibroblast **growth factor** (FGF-2) during odontogenesis of mouse as revealed by immunohistology. Parasagittal sections of mouse embryo head (13-18 day of gestation) containing various stages of developing tooth were incubated with a polyclonal anti-FGF-2 **antibody** and positive binding was evidentiated by using **Streptavidin**-Biotin complex-HRP system and AEC staining. We observed no FGF-2 staining at the

dental lamina stage. At the bud stage slight staining is seen, limited to some epithelial cells. The intensity of the staining increases at the cap stage. In the bell stage, the stellate reticulum cells stain intensely. Later, odontoblasts and the dentin matrix stain deeply; but the epithelial cells stain faint. The extra cellular matrix of the dentin and dental papilla stain very intense but the enamel matrix is found negative. These results indicate the participation of FGF-2 in differentiation rather than in proliferation of tooth-forming cells. In particular, it appears that FGF-2 participates in odontoblast differentiation and in dentin matrix deposition. The spatio-temporally specific distribution pattern of FGF-2 in developing mouse tooth reported here emphasizes the importance of FGF-2 in mammalian odontogenesis.

L27 ANSWER 17 OF 68 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 4
1998:515062 Document No. 129:274359 Development of a CD28/CD86 (B7-2) binding assay for high throughput screening by homogeneous time-resolved **fluorescence**. Mellor, Geoffrey W.; Burden, M. Neil; Preaudat, Marc; Joseph, Yvonne; Cooksley, Susan B.; Ellis, Jonathan H.; Banks, Martyn N. (Lead Discovery Unit, Glaxo Wellcome Research and Development, Stevenage, UK). J. Biomol. Screening, 3(2), 91-99 (English) 1998. CODEN: JBISF3. ISSN: 1087-0571. Publisher: Mary Ann Liebert, Inc..

Date

AB CD28 has been demonstrated to provide the major costimulatory signal for CD4-pos. T cells. Ligation with its natural ligands CD80 (B7-1) and CD86 (B7-2) leads to signals during activation that are required for the prodn. of **interleukin**-2, and this process has been implicated in the regulation of T-cell anergy and programmed cell death. This article describes the assay development, assay validation, and primary screening for small mol. antagonists of this interaction, which could be potential drug candidates. The assay uses homogeneous time-resolved **fluorescence** based on energy transfer from excited europium ions to cross-linked allophycocyanin, which then subsequently emits a **fluorescent** signal. An "indirect" approach was taken, whereby the cross-linked allophycocyanin (XL665) is covalently linked to an antihuman **antibody** that binds to a human Ig domain fused to CD28. The CD86 that is expressed as a fusion **protein** with a rat Ig domain is bound to biotinylated sheep antirat **antibody**, which is complexed with **streptavidin**-europium cryptate. This "cassette" format facilitates the development of related assays using CTLA-4 in place of CD28 and/or CD80 in place of CD86, allowing easy detn. of the selectivity of active compds. When the CD28 and CD86 are in close proximity (i.e., bound), there is a specific time-resolved emission at 665 nm that is largely absent in either unbound partner. Expts. to optimize the reagent concns., incubation time, solvent effects and quench effects by colored compds. are discussed, as are the results from robustness testing and data from primary screening.

L27 ANSWER 18 OF 68 MEDLINE DUPLICATE 5
1998352968 Document Number: 98352968. Receptors for BPH **growth factors** are located in some neuroendocrine cells. Iwamura M; Koshiba K; Cockett A T. (Department of Urology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan.)PROSTATE. SUPPLEMENT, (1998) 8 14-7. Journal code: ANH. ISSN: 1050-5881. Pub. country: United States. Language: English.

AB BACKGROUND: Prostatic neuroendocrine (NE) cells play an important role in

the growth and differentiation of the prostate. We are still unable to characterize the exact mechanisms which lead to interactions between the epithelial cell and the NE cell. We offer several interactions generated by the NE cells, and speculate on some actions of selected NE cells. METHODS: We used thin sections of prostatic tissue made from 20 radical prostatectomies. Our team used validated rabbit **polyclonal antibodies** which were raised against human EGFR and C-erb B-2, using the **streptavidin**-peroxidase conjugate method. RESULTS: A strong immunoreactivity was noted with both **antibodies** in the cytosol of some NE cells. These cells had a dendritic appearance, and they were located in the acini and ducts of small-to-moderate-sized prostatic glands. Double immunostaining revealed the colocalization of both antigens with chromogranin A (CgA), a polypeptide that is expressed by NE cells. Of interest was the finding that EGFR and C-erb B-2 were colocalized as well as independently expressed by separate populations of NE cells. CONCLUSIONS: We conclude that NE cells may be regulated by the HER **protein** family, probably in a ligand-specific fashion. This is a revised report which identifies a pathway regulating NE cells, and their interactions with epithelial cells.

L27 ANSWER 19 OF 68 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.

1998252808 EMBASE Receptors for BPH **growth factors** are located in some neuroendocrine cells. Iwamura M.; Koshiba K.; Cockett A.T.K.. Dr. A.T.K. Cockett, Department of Urology, Box 656, Univ. of Rochester Medical Center, 601 Elmwood Ave., Rochester, NY 14642, United States. Prostate 36/SUPPL. 8 (14-17) 1998.
Refs: 7.
ISSN: 0270-4137. CODEN: PRSTDS. Pub. Country: United States. Language: English. Summary Language: English.

AB BACKGROUND. Prostatic neuroendocrine (NE) cells play an important role in the growth and differentiation of the prostate. We are still unable to characterize the exact mechanisms which lead to interactions between the epithelial cell and the NE cell. We offer several interactions generated by the NE cells, and speculate on some actions of selected NE cells. METHODS. We used thin sections of prostatic tissue made from 20 radical prostatectomies. Our team used validated rabbit **polyclonal antibodies** which were raised against human EGFR and C-erb B-2, using the **streptavidin**-peroxidase conjugate method. RESULTS. A strong immunoreactivity was noted with both **antibodies** in the cytosol of some NE cells. These cells had a dendritic appearance, and they were located in the acini and ducts of small-to-moderate-sized prostatic glands. Double immunostaining revealed the colocalization of both antigens with chromogranin A (CgA), a polypeptide that is expressed by NE cells. Of interest was the finding that EGFR and C-erb B-2 were colocalized as well as independently expressed by separate populations of NE cells. CONCLUSIONS. We conclude that NE cells may be regulated by the HER **protein** family, probably in a ligand-specific fashion. This is a revised report which identifies a pathway regulating NE cells, and their interactions with epithelial cells.

L27 ANSWER 20 OF 68 CAPLUS COPYRIGHT 1999 ACS

1997:502910 Document No. 127:140575 Modified avidin-type molecules as targeting agents for the liver and cells of the reticuloendothelial system. Schechter, Bilha; Arnon, Ruth; Wilchek, Meir (Yeda Research and Development Co. Ltd., Israel; Mcinnis, Patricia, A.; Schechter, Bilha;

Arnon, Ruth; Wilchek, Meir). PCT Int. Appl. WO 9722879 A1 19970626, 66 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 96-US20333 19961220. PRIORITY: IL 95-116500 19951221.

AB The present invention relates to avidin-type mols. having 2,4,6-trinitrophenyl or lactosyl groups or being complexed with an **antibody** specific to the avidin-type mol., which shifts the biodistribution pattern in tissues and organs to the liver, where these mols. accumulate at high levels over several days. These modified avidin-type mols. provide a means for delivery of diagnostic and therapeutic agents, including radionuclides to the liver and cells of the reticuloendothelial system (RES) for diagnosing and treating hepatic disorders and disorders of the RES.

L27 ANSWER 21 OF 68 CAPLUS COPYRIGHT 1999 ACS

1997:429603 Document No. 127:46043 Method and probe for detecting a single-stranded target nucleic acid sequence by hybridization and digestion of unhybridized single-stranded nucleic acid. Russek, Shelley J.; Farb, David H. (Trustees of Boston University, USA; Russek, Shelley J.; Farb, David H.). PCT Int. Appl. WO 9719192 A1 19970529, 48 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 96-US18615 19961120. PRIORITY: US 95-7381 19951120.

AB In the method of the invention, nucleic acid in a sample is made single-stranded and exposed to a probe contg. a sequence complementary to a target sequence under hybridizing conditions. Any single-stranded nucleic acid present after hybridization is digested by addn. of nuclease.
The target sequence is present if the probes are intact after the digestion of the single strands of the sample. The probes have either member in a specific binding pair (such as biotin-avidin) attached to the probe distal form a **detectable label**; after digestion the probe is immobilized by the other member of the binding pair, or 2 **fluorescent** labels affecting each other if they are in each others vicinity. Immunoassays for **protein** antigens can be carried out by a variant of the method in which an **antibody** is conjugated to a nucleic acid complementary to a specific probe.

L27 ANSWER 22 OF 68 CAPLUS COPYRIGHT 1999 ACS

1997:324386 Document No. 126:290391 **Streptavidin** peptides and **proteins** with altered physical properties for analysis and purification purposes and nucleic acids encoding them. Sano, Takeshi; Cantor, Charles R.; Vajda, Sandor; Reznik, Gabriel O.; Smith, Cassandra L.; Pandori, Mark W. (Trustees of Boston University, USA). PCT Int. Appl. WO 9711183 A1 19970327, 90 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN,

AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 96-US5169 19960410. PRIORITY:
US 95-420010 19950411; US 95-3687 19950918.

AB The present invention relates to **streptavidin proteins** and peptides having altered phys. properties such as an increased stability or increased or decreased **affinity** for binding biotin. The invention also relates to methods for the detection, identification, sepn., and isolation of targets using **streptavidin proteins** or peptides. **Streptavidin** with increased or reduced **affinity** allows for the use of the **streptavidin** -biotin coupling systems wherein it is necessary to remove one or the other of the binding partners. Such systems are useful for the purifn. of functional **proteins** and viable cells. The invention also relates to nucleic acids which encode these **streptavidin proteins** and peptides and to recombinant cells such as bacteria, yeast and mammalian cells which contain these nucleic acids.

L27 ANSWER 23 OF 68 CAPLUS COPYRIGHT 1999 ACS
1997:215792 Document No. 126:196103 Viral vectors with modified surfaces and their use in the targetting of transforming DNA to specific cell types. Valerio, Domenico; Van Beusechem, Victor Willem (Introgene B.V., Neth.; Valerio, Domenico; Van Beusechem, Victor Willem). PCT Int. Appl. WO 9705266 A1 19970213, 52 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 96-NL302 19960725. PRIORITY: EP 95-202040
19950725.

AB A method of delivering nucleic acids to a specific cell type using a viral vector with a modified viral surface is described. The virus has a modified capsid or envelope **protein** that presents a ligand for an intermediate **protein**. The intermediate **protein** has two **binding sites**, one is for the new moiety presented on the virus and the other is for a moiety presented on the surface of the target cell. The virus becomes bound to the target cell surface by this intermediate **protein**. The use of this bipartite intermediate **protein** means that a new virus does not have to be designed for each new cell type. The viral surface is modified to minimize or eliminate virus binding to its natural target. The moiety on the virus can be for example an Ig binding moiety (e.g. capable of binding to a Fc fragment, **protein** A, **protein** G, FcR or an anti-Ig **antibody**), or biotin, avidin or **streptavidin**. The outer membrane or capsid of the virus may contain a substance which mediates entrance of the gene delivery vehicle into the target cell. Due to the specificity of the ligand, the high **affinity** of the binding pair and to the inability of the gene delivery vehicle to be targeted when used alone, the universality of the method for gene delivery, together with its high cell type selectively can easily be achieved by the use of various targeting conjugates. The development of a Moloney murine leukemia virus with an Fc.gamma.RI Ig-binding domain on the viral surface is described.

L27 ANSWER 24 OF 68 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 98-008044 [01] WPIDS
CR 98-361794 [31]
AB US 5688966 A UPAB: 980805
Sulpho-indolenine compounds of formula (I) are new.
X = C(Me)2, O or S; Z = linking group, and
A = enzyme substrate or member of a specific binding pair.
Also claimed are tyramide derivative sulpho-indocyanine dyes of formula (II').
m = 1-3.
USE - (I) are intermediates for **fluorescent** sulpho-indocyanine dyes (II) (including (II')), which are useful as **fluorescent** labels.
Specific binding pairs in A include antigen/**antibody**, hapten/**anti-hapten**, biotin/avidin, biotin/**streptavidin**, folic acid/folate binding **protein** and complementary probe nucleic acids.
Fluorescent labels in general are used e.g. with **antibodies**, DNA probes, biochemical analogues, lipids, drugs, **cytokines**, cells or polymers.
Tyramide-containing (II), e.g. (II'), can be used as reporter substrates in an enzyme-based signal amplification called catalysed reporter deposition ('CARD'; see US5196306).
ADVANTAGE - (I)), and therefore (II), are already linked to an enzyme substrate or a member of a specific binding pair, rather than containing a reactive group which will covalently attach to a target molecule.
Dwg.0/1

L27 ANSWER 25 OF 68 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
97127814 EMBASE Document No.: 1997127814. Clinical and prognostic significance of the expression of the c-erbB-2 and c-erbB-3 oncoproteins in primary and metastatic malignant melanomas and breast carcinomas.
Bodey B.; Bodey B. Jr.; Groger A.M.; Luck J.V.; Siegel S.E.; Taylor C.R.; Kaiser H.E.. Dr. B. Bodey, 15745 Saticoy Street, Van Nuys, CA 91406, United States. Anticancer Research 17/2 B (1319-1330) 1997.
Refs: 186.
ISSN: 0250-7005. CODEN: ANTRD4. Pub. Country: Greece. Language: English. Summary Language: English.
AB Several **growth factors** and proto-oncogenes play a leading regulatory role during human carcinogenesis. In this systematic immunocytochemical study we observed the expression (overexpression) of the c-erbB-2 and c-erbB-3 oncoproteins in 30 primary cutaneous malignant melanomas (CMMs), 10 already metastasized malignant melanomas (MMMs) and 15 lymph-node negative breast carcinomas (BCs). Both oncoproteins were expressed as a result of either oncogene amplification or post-translational stabilization. c-erbB-2 alone is unable to bind neuregulins, but it is able to act as a pan c-erbB receptor subunit. Heterodimerization between cerbB-2 and c-erbB-3 is required to initiate neuregulin directed signal transduction. We employed an indirect, four step **streptavidin**-biotin conjugated immunocytochemical technique for antigen defection. The visualization of the primary antigen-**antibody** reaction was carried out with alkaline phosphatase or immunoperoxidase labeling and the use of the appropriate **enzymatic** substrates. The presence of c-erbB-2 oncoprotein was detected in 12/30 CMMs, 8/10 MMMs and 6/15 BCs, while c-erbB-3 was identified in 14/30 CMMs, 7/10 MMMs and 6/15 BCs. The intensity of the cell membrane localized

immunoreactivity was observed to be greater when the c-erbB-2 oncoprotein was targeted (A, AB and B). The c-erbB-3 oncoprotein was also detected in the cytoplasm with medium intensity (B, BC and C). Unfortunately, little is known concerning the range of oncoprotein overexpression after formalin
fixation and paraffin embedding. We demonstrated overexpression localized to several cell clones within the oncoprotein positive population of malignant cells. The immunocytochemically defined extent of expression of both oncoproteins was between 10-40% (+ to ++) of the total cell population in the malignant melanomas and 20-35% (++) of the total cell population in the BCs. In conclusion a) the results of the present study demonstrate the presence of c-erbB-2 and c-erbB-3 oncoprotein expression (overexpression) in melanoma and breast carcinoma and b) oncogene receptor
directed immunotherapy, as part of a more individualized anti-cancer treatment, represents a potentially valuable targeted treatment for the future.

L27 ANSWER 26 OF 68 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
97237159 EMBASE Document No.: 1997237159. Detection of serum insulin-like **growth factor** binding **proteins** on Western
ligand blots by biotinylated IGF and enhanced chemiluminescence. De Beeck L.O.; Verlooy J.E.A.; Van Buul-Offers S.C.; Du Caju M.V.L.. L.O. De Beeck,
Department of Pediatrics, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium. Journal of Endocrinology 154/2 (R1-R5) 1997. Refs: 16.
ISSN: 0022-0795. CODEN: JOENAK. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB A novel procedure for the detection of IGF binding capacity of IGFBPs on Western ligand blots (WLB) was developed using biotinylated IGFs as probes. The biotinylated IGF-IGFBP complexes were visualized by **streptavidin**- horseradish peroxidase and enhanced chemiluminescence (ECL). The procedure was found to be faster and more efficient than the conventional method with iodinated IGFs. In normal human serum a predominant doublet at 38-42 kDa and five smaller bands at 35, 34, 30, 28 and 24 kDa were detected by both methods, whereas two additional bands at 26 and 16 kDa became visible with the ECL method. In pregnancy serum only one single faint band at 30 kDa could be detected by the iodinated method. In contrast, the ECL method revealed five other bands at 42, 34, 28, 26 and 16 kDa. Besides the 38-42 kDa doublet, the 30 and 16 kDa bands reacted strongly with anti-IGFBP-3 **antibodies** in Western immunoblotting (WIB) and therefore were related to IGFBP-3 fragments. The technical advantages of this ECL method include an extremely short exposure time to the radiographic film and a long stability of the probe. In addition, the ECL method is a non-radioactive method, making radioprotection and radioactive waste removal unnecessary.

L27 ANSWER 27 OF 68 CAPLUS COPYRIGHT 1999 ACS
1997:121366 Document No. 126:130594 Improved delivery of diagnostic and therapeutic agents to a target site. Griffiths, Gary L.; Hansen, Hans J.;
Govindan, Serengulam (Immunomedics, Inc., USA). PCT Int. Appl. WO 9640245
A1 19961219, 41 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 96-US8696 19960607. PRIORITY: US 95-486166 19950607.

AB An in vivo method for delivering a diagnostic or therapeutic agent to a target site in a mammal, wherein a targeting species including a targeting moiety and a diagnostic or therapeutic agent or a **binding site** for a subsequently administered diagnostic or therapeutic agent conjugate, the targeting moiety having a primary **binding site** whereby it specifically binds to the target, is administered and allowed to accrete at the target site, is improved by injecting into the circulatory system of the mammal a clearing agent that binds to the primary **binding site** of the targeting species, whereby non-localized primary targeting species is cleared from circulation. The method is esp. useful in pretargeting methods because the clearing agent does not remove the primary targeting species or block secondary **binding sites** of the primary targeting species. Described are prepn. of **streptavidin**/anti-carcinoembryonic antigen **antibody** (IMMU-14) conjugate, prepn. of biotin-carborane-dextran conjugate, prepn. of yttrium-90-labeled p-[5-(biotinamido)pentyl(amino)thioureayl]-2-benzyl-diethylenetriaminepentaacetic acid (BPD), in vivo localization of Y-90-BPD to pretargeted **streptavidin**-IMMU-14, localization of biotin-carborane-dextran to pretargeted **streptavidin**-IgG, delivery of In-111 to tumor xenografts using the invented pretargeting protocol, etc.

L27 ANSWER 28 OF 68 CAPLUS COPYRIGHT 1999 ACS
1996:290658 Document No. 124:315061 Polyspecific immunoconjugates and **antibody** composites for targeting the multidrug resistant phenotype. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 9604313 A1 19960215, 83 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 95-US9491 19950801. PRIORITY: US 94-286430 19940805.
AB Polyspecific immunoconjugates and **antibody** composites that bind a multidrug transporter **protein** and an antigen assocd. with a tumor or infectious agent are used to overcom the multidrug resistant phenotype. These immunoconjugates and composites also can be used diagnostically to det. whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents. In example, polyspecific immunoconjugates contg. G-CSF, 90Yttrium, doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen bispecific **antibody** were prepd. for colon cancer treatment. 99mTc- and 111Indium-labeled polyspecific immunoconjugate targeted to multidrug resistant Pseudomonas aeruginosa were also prepd.

L27 ANSWER 29 OF 68 CAPLUS COPYRIGHT 1999 ACS
1996:548731 Document No. 125:242411 Composition for introducing nucleic acid complexes into higher eukaryotic cells. Curiel, David T.; Birnstiel, Max L.; Cotten, Matthew; Wagner, Ernst; Zatloukal, Kurt; Plank, Christian; Oberhauser, Berndt; Schmidt, Walter G. M. (Boehringer Ingelheim International GmbH, Germany; Genentech, Inc.). U.S. US 5547932 A 19960820, 119 pp. Cont.-in-part of U.S. Ser. No. 827, 103, abandoned. (English). CODEN: USXXAM. APPLICATION: US 92-948357 19920923. PRIORITY:

US 91-768039 19910930; US 91-767788 19910930; US 92-827103 19920130; US 92-827102 19920130; US 92-864759 19920407; US 92-937788 19920902.

AB A compn. for the transfection of higher eukaryotic cells, comprising complexes of nucleic acid, a substance having an **affinity** for nucleic acid, and optionally an internalizing factor, contains an endosomolytic agent, e.g., a virus or virus component, which may be conjugated. The endosomolytic agent, which is optionally part of the nucleic acid complex, is internalized into the cells together with the complex and releases the contents of the endosomes into the cytoplasm, thereby increasing the gene transfer capacity. Examples are given of transfecting tumor cells with genes that encode immunomodulating substances, e.g., **cytokines**. Pharmaceutical prepns., transfection kits, and methods for introducing nucleic acids into higher eukaryotic cells by treating the cells with the compn. are also disclosed.

L27 ANSWER 30 OF 68 CAPLUS COPYRIGHT 1999 ACS

1997:42008 Document No. 126:57123 Method for analyzing biological active substances. Suzuki, Osamu; Sasaki, Naokazu; Ichihara, Tatsuo; Okada, Sanae (Nisshinbo Industries, Inc., Japan). Eur. Pat. Appl. EP 747703 A2 19961211, 22 pp. DESIGNATED STATES: R: DE, FR, GB. (English). CODEN: EPXXDW. APPLICATION: EP 96-304158 19960605. PRIORITY: JP 95-143715 19950609.

AB A method is provided, comprising the steps of reacting a biol. active first substance immobilized on a carrier with a second substance capable of specifically binding the first substance, and detecting a non-bound part of the second substance or a bound part of the second substance indirectly bound to the carrier through binding between the first and second substances so that the first substance or the second substance in a sample is analyzed, wherein the carrier carries a compd. having 2-100 carbodiimide groups, and the first substance is immobilized on the carrier through the carbodiimide groups so that the active substance such as **protein** and nucleic acid is bound to the carrier conveniently, efficiently, and tightly.

L27 ANSWER 31 OF 68 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.

96249964 EMBASE Document No.: 1996249964. Localization of vascular endothelial **growth factor** in human retina and choroid.
Lutty G.A.; McLeod S.; Merges C.; Diggs A.; Plouet J.. 170 Woods Research Building, John Hopkins University, 600 N Wolfe St,Baltimore, MD 21287-9115, United States. Archives of Ophthalmology 114/8 (971-977) 1996.
ISSN: 0003-9950. CODEN: AROPAW. Pub. Country: United States. Language: English. Summary Language: English.

AB Objective: To examine the distribution and relative levels of vascular endothelial **growth factor** (VEGF) in the nondiabetic and preproliferative diabetic human retina and choroid. Methods: Immunohistochemical localization was performed on frozen sections from cryopreserved postmortem human tissue using a **polyclonal antibody** against VEGF and a **streptavidin** peroxidase system. Eyes from 5 subjects without diabetes and 8 subjects with diabetes were examined and analyzed using a 7-point immunohistochemical grading system. Results: In subjects without diabetes, weak or no VEGF immunoreactivity was associated with retinal blood vessels. In subjects with diabetes, we found significantly increased immunoreactivity in the retinal vascular endothelium and blood vessel walls. Vascular endothelial **growth factor** immunoreactivity was also associated with intravascular leukocytes in subjects with and without diabetes. In the

choroid of subjects without diabetes, immunoreactivity was almost exclusively associated with intravascular leukocytes, whereas in diabetic subjects, immunoreactivity was localized within choriocapillaris endothelium, choroidal neovascular endothelium, and migrating retinal pigment epithelium cells. Conclusions: The observed increase in VEGF immunoreactivity in the diabetic retina and choroid suggests that VEGF may contribute to 2 well-documented events during retinopathy: increased vascular permeability and angiogenesis.

L27 ANSWER 32 OF 68 CAPLUS COPYRIGHT 1999 ACS
1996:161185 Document No. 124:197760 Photocleavable agents and conjugates for the detection and isolation of biomolecules.. Rothschild, Kenneth J.; Sonar, Sanjay M.; Olejnik, Jerzy (USA). PCT Int. Appl. WO 9531429 A1 19951123, 149 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 95-US5555 19950511. PRIORITY: US 94-240511 19940511; US 94-345807 19941122.
AB This invention relates to agents and conjugates that can be used to detect and isolate target components from complex mixts. such as nucleic acids from biol. samples, cells from bodily fluids, and nascent **proteins** from translation reactions. Agents comprise a detectable moiety bound to a photoreactive moiety. Conjugates comprise agents coupled to substrates by covalent bonds which can be selectively cleaved with the administration of electromagnetic radiation. Target substances labeled with detectable mols. can be easily identified and sepd. from a heterologous mixt. of substances. Exposure of the conjugate to radiation releases the target in a functional form and completely unaltered. Using photocleavable mol. precursors as the conjugates, label can be incorporated into **macromols.**, the nascent **macromols.** isolated, and the label completely removed. The invention also relates to targets isolated with these conjugates which may be useful as pharmaceutical agents or compns. that can be administered to humans and other mammals. Useful compns. include biol. agents such as nucleic acids, **proteins**, lipids and **cytokines**. Conjugates can also be used to monitor the pathway and half-life of pharmaceutical compns. in vivo and for diagnostic, therapeutic and prophylactic purposes. The invention also relates to kits comprised of agents and conjugates that can be used for the detection of diseases, disorders and nearly any individual substance in a complex background of substances.

L27 ANSWER 33 OF 68 CAPLUS COPYRIGHT 1999 ACS
1995:861337 Document No. 123:246795 Self-assembling multimeric nucleic acid constructs for diagnostic, therapeutic, and other uses. Cantor, Charles R.; Niemeyer, Christof M.; Smith, Cassandra L.; Sano, Takeshi; Hnatowich, Donald J.; Rusckowski, Mary (Trustees of Boston University, USA; University of Massachusetts Medical Center). PCT Int. Appl. WO 9520320 A1 19950803, 45 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 95-US864 19950127. PRIORITY: US 94-189448 19940131.
AB The invention is directed to constructs and compns. contg. multimeric

forms of nucleic acid. Multimeric nucleic acids comprise single-stranded nucleic acids attached via biotin to **streptavidin** and bound with a functional group. The constructs can be utilized in vivo to treat or identify diseased tissue or cells. Repeated administrations of multimeric nucleic acid compns. produce a rapid and specific amplification of nucleic acid constructs and their attached functional groups. For treatment purposes, functional groups may be toxins, **radioisotopes**, genes or enzymes. Diagnostically, labeled multimeric constructs may be used to identify specific targets in vivo or in vitro. Multimeric nucleic acids may also be used in nanotechnol. and to create self-assembling polymeric aggregates such as membranes of defined porosity, microcircuits and many other products.

L27 ANSWER 34 OF 68 MEDLINE DUPLICATE 6
95348515 Document Number: 95348515. Biotinylation of **interleukin**-2 (IL-2) for flow cytometric analysis of IL-2 receptor expression. Comparison of different methods. De Jong M O; Rozemuller H; Bauman J G; Visser J W. (Department of Hematology, Erasmus University Rotterdam, Netherlands..)JOURNAL OF IMMUNOLOGICAL METHODS, (1995 Jul 17) 184 (1) 101-12. Journal code: IFE. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB The main prerequisites for the use of biotinylated ligands to study the expression of **growth factor** receptors on heterogeneous cell populations, such as peripheral blood or bone marrow, by flow cytometric methods, are that the biotinylated ligand retains its binding ability and that binding of the biotinylated ligand to the receptor does not inhibit the subsequent interaction of biotin with **fluorescently** tagged avidin or **streptavidin**. Using **interleukin**-2 (IL-2), we compared the usefulness of various biotinylation reagents, NHS-biotin, S-NHS-biotin, S-NHS-LC-biotin, DBB and photobiotin, and developed optimal biotinylation conditions for the preparation of biologically active biotin-labeled IL-2 and the detection of IL-2 receptor expressing cells by flow cytometry. As determined by spot blot analysis, biotinylation of IL-2 was most efficient at the highest biotin-to-**protein** (B:P) ratio used. At a B:P ratio of 100, most of the biological activity of IL-2 was retained when S-NHS-LC-biotin was used. In contrast, most of the biological activity of IL-2 samples that were labeled with NHS-biotin or photobiotin was lost under these conditions. Biotin-labeled IL-2 preparations were tested in order to detect IL-2 receptors on IL-2 dependent CTLL-2 cells by flow cytometry after sequential staining with the biotinylated IL-2 and **fluorescence** tagged **streptavidin**. A high B:P ratio generally resulted in a high specific **fluorescence** intensity of the cells, particularly when S-NHS-LC-biotin was used as the biotinylation reagent. Biotin-IL-2 could also be used to detect IL-2 receptors expressed by lymphocytes in peripheral blood and bone marrow. Comparison of staining of lymphocytes with biotinylated IL-2 and an **antibody** against the IL-2 receptor alpha chain demonstrated that only a subset of the cells that showed a strong **fluorescence** signal after staining with biotinylated IL-2 expressed high numbers of the IL-2 receptor alpha chain. This is in agreement with the expression of functional IL-2 receptors on resting T cells and NK cells which do not express the alpha chain. After

stimulation with PHA, virtually all lymphocytes expressed the alpha chain, whereas only part of these cells showed a strong **fluorescence** signal after staining with biotin-IL-2, while the rest of the cells had very low numbers of IL-2 **binding sites**. Our results demonstrate that, in addition to staining individual receptor subunits with **antibodies**, staining with biotinylated IL-2 is a useful indicator of functional IL-2 receptor expression.

L27 ANSWER 35 OF 68 CAPLUS COPYRIGHT 1999 ACS
1994:404522 Document No. 121:4522 Bridge immunoassay. LaMotte, George B., III (Ciba Corning Diagnostics Corp., USA). U.S. US 5296347 A 19940322, 24 pp. Cont. of U.S. Ser. No. 653,024, abandoned. (English). CODEN: USXXAM. APPLICATION: US 93-14092 19930204. PRIORITY: US 91-653024 19910208.

AB Disclosed is a bridge immunoassay, which employs a primary free soln. analyte/receptor binding reaction, for example, in a sandwich-type format (two or more analyte receptors), in a competitive format (single analyte receptor), or in a related immunoassay format, and a universal solid phase and capture system. The universal capture system comprises a first receptor bound to a solid phase and a bridge receptor (a second receptor) which functions both as a ligand for the bound first receptor and as a receptor for a ligand conjugated to a sample analyte receptor (a third receptor). The bridge receptor is used to immobilize the immunocomplexes formed free in soln. by linking them to the bound first receptor. The universal capture system can be used for assays for any analyte as the bridge receptor binds to a ligand, for example, a hapten or binding **protein**, conjugated to the sample analyte receptor. Methods, compns. and test kits for such bridge immunoassays are provided. A sandwich EIA for serum c-erbB-2 **protein** is described which uses both mouse anti-c-erbB-2 monoclonal **antibodies** conjugated to either the hapten FITC or to horseradish peroxidase, c-erbB-2 calibrators and controls, a biotinylated mouse monoclonal **antibody** to FITC as the bridge receptor, and polystyrene tubes coated with **streptavidin**.

L27 ANSWER 36 OF 68 MEDLINE DUPLICATE 7
94321139 Document Number: 94321139. Immunohistochemical localization of transforming **growth factor**-beta 1, -beta 2, and -beta 3 latency-associated peptide in human cornea. Nishida K; Kinoshita S; Yokoi N; Kaneda M; Hashimoto K; Yamamoto S. (Department of Ophthalmology, Kyoto Prefectural University of Medicine, Japan..)INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, (1994 Jul) 35 (8) 3289-94. Journal code: GWI. ISSN: 0146-0404. Pub. country: United States. Language: English.

AB PURPOSE. To study spatial distribution of TGF-beta isoforms (TGF-beta 1, -beta 2, and -beta 3) in the human cornea and to elucidate their biologic roles in corneal maintenance. METHODS. Frozen sections obtained from eight human autopsy eyes were placed on gelatin-coated slides. After blocking of nonspecific **binding sites**, the slides were incubated with rabbit **polyclonal antibody** to the latency-associated peptide (LAP) region of human TGF-beta 1, TGF-beta 2, and TGF-beta 3 precursors, followed by the incubation with biotinylated swine anti-rabbit IgG. Subsequently, a **streptavidin**-labeled alkaline phosphatase technique was used. RESULTS. In the corneal region, beta 1-LAP **antibody** did not stain either epithelium or stroma, beta 2-LAP **antibody** stained all epithelial cell layers and the corneal stroma, and beta 3-LAP **antibody** stained the

subepithelial region alone. The staining pattern in the limbal region was almost the same as in the corneal region, except in the limbal stroma, which was stained with beta 1-LAP **antibody** in three of eight samples. In the trabecular meshwork, all samples showed clear staining with beta 2-LAP **antibody**, whereas beta 1-LAP and beta 2-LAP **antibody** stained faintly in five of eight and four of eight samples, respectively. CONCLUSION. beta 2-LAP was found in the corneal epithelium and stroma and beta 3-LAP in the subepithelial region, suggesting that TGF-beta 2 and TGF-beta 3 may play essential roles in normal corneal epithelial maintenance in vivo.

L27 ANSWER 37 OF 68 MEDLINE DUPLICATE 8
94255980 Document Number: 94255980. Overexpression of human epidermal **growth factor** receptor and c-erbB-2 by neuroendocrine cells in normal prostatic tissue. Iwamura M; di Sant'agnese P A; Wu G; Benning C M; Cockett A T; Gershagen S. (Department of Urology, University of Rochester Medical Center, New York.)UROLOGY, (1994 Jun) 43 (6) 838-43.
Journal code: WSY. ISSN: 0090-4295. Pub. country: United States. Language:
English.
AB OBJECTIVE. It has been suggested that prostatic neuroendocrine (NE) cells play an important role in the growth and differentiation of the prostate by secreting various neuropeptides and serotonin. However, the mechanism by which NE cells themselves are regulated is virtually unknown. In the present study we evaluated the expression of the human epidermal **growth factor** receptor (EGFR) family (HER) in prostatic NE cells. METHODS. Formalin-fixed, paraffin-embedded tissue sections from twenty radical prostatectomy specimens were immunostained with validated rabbit **polyclonal antibodies** raised against human EGFR and c-erbB-2, using the **streptavidin**-peroxidase enzyme conjugate method. RESULTS. A strong immunoreactivity was observed with both **antibodies** in the cytosol of a few epithelial cells. These cells frequently had a dendritic appearance and were located in the acini and ducts. The EGFR-positive cells were predominant in most cases. Double immunostaining revealed the colocalization of both antigens with chromogranin A, a polypeptide that is expressed by most NE cells. Moreover, EGFR and c-erbB-2 appeared to be colocalized as well as independently expressed by different subpopulations of NE cells. CONCLUSIONS. The results suggest that prostatic NE cells might be regulated by the HER **protein** family, probably, in a ligand-specific fashion. This is the first report identifying a potential pathway regulating prostatic NE cells.

L27 ANSWER 38 OF 68 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
94373638 EMBASE Document No.: 1994373638. Tissue distribution of the human MDR3 P-glycoprotein. Smit J.J.M.; Schinkel A.H.; Mol C.A.A.M.; Majoor D.; Mooi W.J.; Jongsma A.P.M.; Lincke C.R.; Borst P.. Netherlands Cancer Institute, Plesmanlaan 121,1066 CX Amsterdam, Netherlands. Laboratory Investigation 71/5 (638-649) 1994.
ISSN: 0023-6837. CODEN: LAINAW. Pub. Country: United States. Language: English. Summary Language: English.
AB BACKGROUND: P-glycoproteins (Pgps) belong to a family of well conserved plasma membrane **proteins** with two members in humans: MDR1 and MDR3. The MDR1 Pgp can transport drugs; the murine homologue of MDR3, mdr2, was recently shown by us to be involved in transport of the phospholipid phosphatidylcholine (lecithin) into bile. EXPERIMENTAL DESIGN: We have determined the MDR3 mRNA levels in a panel of human tissues by RNase protection. We have also generated **polyclonal antibodies** specific for the MDR3 Pgp. Detection of the MDR3 Pgp in human tissues with these **antibodies** was by a

streptavidin-ABC procedure. RESULTS: The RNase protection results show that expression of the MDR3 gene has a more restricted distribution than that of MDR1. A high level of MDR3 mRNA was detected in the liver and in low levels in the adrenal gland, heart, striated muscle, spleen, and tonsil. In all of these tissues, some of the previously described splice variants of MDR3 were abundantly expressed. No indications were found for a tissue- specific regulation of alternative splicing of the MDR3 pre-mRNA. Two MDR3 Pgp-specific **antibodies** stained the bile canalicular membrane of hepatocytes across the entire liver lobule. No staining was found in the epithelial cells of the bile ductules and gall bladder, indicating that the staining at these sites with C219, a monoclonal **antibody** that recognizes both MDR1 and MDR3 Pgp, (mainly) represents the MDR1 Pgp. No MDR3 was detected by specific **antibodies** in the adrenal gland, spleen, and muscle. Since no staining was reported with MDR1-specific **antibodies** in muscle either, our results indicate that the C219 staining in some fibers of striated muscle represents a cross- reaction with another **protein** . One of the human MDR3-specific **antibodies** cross-reacted with the highly homologous mouse mdr2 Pgp. Staining with this **antibody** showed that the distribution of this **protein** in mouse liver and striated muscle is very similar to that of MDR3 Pgp in human tissues. CONCLUSIONS: The highest expression of the MDR3 Pgp was found in liver in the canalicular membranes of hepatocytes. This is in agreement with a role for MDR3 in the transport of phospholipid into bile.

L27 ANSWER 39 OF 68 MEDLINE DUPLICATE 9

95128187 Document Number: 95128187. In vivo biotinylated recombinant **antibodies**: construction, characterization, and application of a bifunctional Fab-BCCP fusion **protein** produced in Escherichia coli. Weiss E; Chatellier J; Orfanoudakis G. (Ecole Superieure de Biotechnologie de Strasbourg, Illkirch, France.)PROTEIN EXPRESSION AND PURIFICATION, (1994 Oct) 5 (5) 509-17. Journal code: BJV. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB We describe a novel vector system suitable for the efficient preparation of in vivo biotinylated **antibody** Fab fragments in Escherichia coli. The previously described pGE20 vector used for the functional expression of truncated heavy (Fd) and light (L) chains of Fab into the bacterial culture medium was modified by inserting the C-terminal 101-amino-acid polypeptide of the biotin carboxyl carrier **protein** subunit of E. coli acetyl-CoA carboxylase (BCCP*). The secreted Fd-BCCP* fusion and L chain **proteins** were found to be disulfide linked and Fab-BCCP* complexes of an IgG1 **antibody** (Mab4) to human **tumor necrosis factor** alpha (TNF) were shown to retain both antigen and **streptavidin**-binding activities. The capacity of the Fab4 linked to BCCP* to bind TNF was identical to that observed with unmodified Fab4. Up to 15% of the expressed hybrids were able to interact with **streptavidin** when exogeneous d-biotin was added into the bacterial culture medium. The Fab4-BCCP* molecules were found to be more efficient than Fab4 linked to an engineered **streptavidin-affinity** tag for the detection of antigen on solid phase. In addition, we show here that the bacterially expressed Fab4-BCCP* complexes, adsorbed to **streptavidin**-agarose beads, can be used for the one-step purification of recombinant TNF by immunoaffinity chromatography.

L27 ANSWER 40 OF 68 MEDLINE

95108523 Document Number: 95108523. Determination of human tumour necrosis factor-alpha (TNF-alpha) by time-resolved immunofluorometric assay. Turpeinen U; Stenman U H. (Helsinki University Central Hospital,

Laboratory, Finland.)SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION, (1994 Oct) 54 (6) 475-83. Journal code: UCP. ISSN: 0036-5513. Pub. country: Norway. Language: English.

AB We have developed a 'sandwich'-type time-resolved immunofluorometric assay (IFMA) for tumour necrosis factor alpha (TNF-alpha) using two monoclonal **antibodies** (mAb) and the **streptavidin**/biotin (SAB) system. In this simple and fast **streptavidin**/biotin IFMA (SAB-IFMA) we used **streptavidin** coated wells to which we added biotinylated mAb for 3 h. After washing, the serum sample was added and incubated for 2 h followed by washing. Another monoclonal europium-labelled tracer **antibody** was added and incubated for 1 h, the wells were washed and the **fluorescence** of Eu measured. We tested various assay conditions in order to optimize the assay for sensitivity and measuring range. Purification of the labelled **antibody** by hydrophobic interaction chromatography was found to be essential to improve sensitivity. With a sample volume of 50 microliters the detection limit was 6 ng l-1 and the analytical range large, i.e. 10,000-fold. The median concentration in serum from healthy subjects was 12 ng l-1 and the reference range < 39 ng l-1. The mean analytical recovery in plasma was 76% and in serum 83%. Separation of serum by gel filtration and assay of TNF-alpha in fractions showed that the assay also measured the high molecular weight (MW) form of TNF-alpha, apparently corresponding to its complex with **soluble receptors.** Advantages of our SAB-IFMA were high sensitivity and low consumption of mAb. The assay performance of the SAB-IFMA was compared to two commercially available enzyme immunoassays also using the SAB system.

L27 ANSWER 41 OF 68 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 10

1994:69729 Document No. 120:69729 An enzyme immunoreceptor assay for the quantitation of insulin-like **growth factor**-I and insulin receptors in bovine muscle tissue. Boge, Annegret; Sauerwein, Helga; Meyer, Heinrich H. D. (Inst. Physiol., TU Muenchen, Freising, 85350, Germany). Anal. Biochem., 216(2), 406-12 (English) 1994. CODEN: ANBCA2. ISSN: 0003-2697.

AB To investigate the regulation of IGF-I and insulin receptor in muscle, a sensitive enzyme immunoreceptor assay (EIRA), which allows for the detn. of both **affinity** and capacity of the receptors, was developed. After solubilization with Triton X-100, receptors were immobilized in microtiter plates using receptor specific monoclonal **antibodies** that recognize the intracellular .beta.-domain of the resp. receptors (clone 17A3 and clone 29B4). The immobilized receptors were labeled with either biotinylated IGF-I or insulin. The bound hormones were detected with a **streptavidin**-horseradish peroxidase technique. The assay had a detection limit of 1 fmol receptor/well. The intraassay variation was 9% (n = 22) for the IGF-I receptor concn. and 12% (n = 22) for the insulin receptor. The interassay variation was 5% (n = 4) for the IGF-I receptor and 10% (n = 4) for the insulin receptor. The relative std. deviations of the dissocn. consts. (Kd) was 26% (n = 7) for the IGF-I receptor and 24% (n = 7) for the insulin receptor. The assay system was used to study the effect of GH treatment upon IGF-I and insulin receptors in bovine skeletal muscle. Three groups of 12 heifers (13 mo old) were treated with either 320 or 640 mg recombinant bovine somatotropin (slow release prepn.) every fortnight for 3 mo. When samples of m. splenius were assayed for IGF-I and insulin receptors, there was no difference between groups for receptor concn. or **affinity**. The amt. of receptor (av. of all groups) was 49 fmol/mg **protein** (insulin) and 97 fmol/mg **protein** (IGF-I). The av. Kd obtained was 0.76 nM (insulin) and 0.66 nM (IGF-I).

L27 ANSWER 42 OF 68 MEDLINE

95235784 Document Number: 95235784. Immunoaffinity measurement of recombinant granulocyte **colony stimulating factor** in patients with chemotherapy-induced neutropenia. Phillips T M. (Immunochemistry Laboratory, George Washington University Medical Center, Washington, D.C. 20037.)JOURNAL OF CHROMATOGRAPHY. B, BIOMEDICAL APPLICATIONS, (1994 Dec 9) 662 (2) 307-13. Journal code: BXL. ISSN: 0378-4347. Pub. country: Netherlands. Language: English.

AB A high-performance immunoaffinity chromatographic technique has been developed for the measurement of recombinant human granulocyte **colony stimulating factor** in human patients receiving this agent, following neutropenia, arising from cancer chemotherapy. The technique employs a short, biocompatible polymer column packed with minute, **antibody**-coated glass beads. This system was applied to the analysis of recombinant human granulocyte **colony stimulating factor** in three different human body fluids. A reasonable degree of correlation was achieved when comparing the immunoaffinity technique to a conventional immunoassay, although the immunoaffinity technique displayed greater specificity.

L27 ANSWER 43 OF 68 MEDLINE DUPLICATE 11

94353379 Document Number: 94353379. Relationship between submandibular gland epidermal **growth factor** and spermatogenesis in C3H mice. Suarez-Quian C A; Oke B O; Radhakrishnan B. (Department of Anatomy and Cell Biology, Georgetown University Medical Center, Washington, DC 20007..)TISSUE AND CELL, (1994 Jun) 26 (3) 285-98. Journal code: VSZ. ISSN: 0040-8166. Pub. country: SCOTLAND: United Kingdom. Language: English.

AB Epidermal **growth factor** (EGF), a potent mitogen produced primarily in the submandibular gland of adult male mice, has been implicated in modulating processes known to be of vital importance in the regulation of spermatogenesis. In the present investigation we demonstrate that submandibular gland EGF from adult male mice is indeed capable of displacing radiolabeled EGF from testicular membranes. Scatchard analysis of this **binding site** reveals that it is of high **affinity** (Kd = 0.77 nM) and low capacity (Bmax = 8.15 fmol/mg **protein**). Cross-linking of 125I-EGF to the identical membrane preparation resulted in the SDS-PAGE/autoradiography identification of a single band at approximately 170 kDa. Next, we examined the cellular distribution of the EGF receptor in the testis using biotin-**streptavidin** immunoperoxidase and employing different antisera probes generated to a conserved sequence of the EGF receptor. The Scatchard and cross-linking data described above, along with the immunocytochemistry results, suggest strongly that there is only one functional **binding site** for EGF in the adult testis and that this receptor is present in Sertoli and Leydig cells.

L27 ANSWER 44 OF 68 MEDLINE

94117856 Document Number: 94117856. A novel and sensitive method for the detection of secreted cell products using time-resolved **fluorescence**. Ruedl C; Wick G; Wolf H. (Institute for General and Experimental Pathology, University of Innsbruck, Medical School, Austria.)JOURNAL OF IMMUNOLOGICAL METHODS, (1994 Jan 12) 168 (1) 61-7. Journal code: IFE. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB A new test has been developed for the quantitative detection of products secreted from isolated cells, based on the use of lanthanide- rather than enzyme-linked **streptavidin**. Used as a label, europium (Eu3+) can be measured with high sensitivity by time-resolved **fluorescence**. The main advantages of this assay are both an increased sensitivity and

measuring range of cell released substances, when compared to the standard "wet" ELISA. Thus, the immunoglobulin secretion rate of 10(5) splenocytes could be easily measured by time-resolved fluoroimmunoassay (TR-FIA), while it remained below the detection limit of the 'wet' ELISA. In contrast to the classical ELISPOT test, this assay does not detect single **antibody** secreting cells (ASC), but would be useful for precise quantification of secreted cell products, such as immunoglobulins, **cytokines, growth factors.**

L27 ANSWER 45 OF 68 CAPLUS COPYRIGHT 1999 ACS
1994:26572 Document No. 120:26572 Intraoperative, intravascular, and endoscopic tumor and lesion detection and therapy. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 9321940 A1 19931111, 49 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 93-US4098 19930506. PRIORITY: US 92-879857 19920506.
AB Medicaments are provided for use in methods of close-range intraoperative, endoscopic and intravascular detection and treatment of lesions, including tumors and nonmalignant lesions (infectious or inflammatory lesions, atherosclerotic plaques, etc.). The medicaments comprise agents labeled with isotopic and nonisotopic agents. Also provided are medicaments for use in methods for detection and treatment of lesions with photodynamic agents and methods of treating lesions with a **protein** conjugated to an agent capable of being activated to emit Auger electrons or other ionizing radiation. Compns. and kits useful in the above methods are also provided. Use of a conjugate of a monoclonal **antibody** [EPB-1 (LL1), which targets atherosclerotic plaques] with Photofrin II for intravascular detection and therapy of a thrombosed myocardia artery is described. Examples of intraoperative and endoscopic tumor detection and intraoperative tumor therapy are also presented.

L27 ANSWER 46 OF 68 MEDLINE
93359728 Document Number: 93359728. A europium fluoroimmunoassay for measuring binding of antigen to class II MHC glycoproteins. Tompkins S M; Rota P A; Moore J C; Jensen P E. (Department of Pathology, Emory University School of Medicine, Atlanta, GA.)JOURNAL OF IMMUNOLOGICAL METHODS, (1993 Aug 9) 163 (2) 209-16. Journal code: IFE. ISSN: 0022-1759.
Pub. country: Netherlands. Language: English.
AB A dissociation-enhanced lanthanide fluoroimmunoassay employing europium-**streptavidin** and time-resolved fluorimetry was developed to measure binding of biotin-labeled peptides to class II MHC **proteins**. Binding of biotin-peptides as measured by this assay was saturable and inhibited in the presence of unlabeled peptide. Background **fluorescence** was minimal and there was a direct relationship between signal and biotin-peptide/class II complex concentration from 1.3 pmol to less than 1 fmol total class II. The sensitivity of the assay and the ability to selectively capture specific class II **proteins** from detergent lysates of cells with solid phase mAb made it possible to measure formation peptide/class II complexes in live APC cultured with biotin-labeled insulin. This assay is expected to be useful for routine measurement of peptide/class II binding and biochemical analysis of Ag processing events.

L27 ANSWER 47 OF 68 CAPLUS COPYRIGHT 1999 ACS
1993:534875 Document No. 119:134875 The time-resolved immunofluorometric

assay (TRIFMA) - diagnostic method of the feature. Missler, Ulrich; Gaida, Ulrike; Li, Hong; Wood, William Graham (Dep. Neurosurg., Med. Univ. Luebeck, Luebeck, Germany). Proc. SPIE-Int. Soc. Opt. Eng., 1885(Proceedings of Advances in Fluorescence Sensing Technology, 1993), 186-98 (English) 1993. CODEN: PSISDG. ISSN: 0277-786X.

AB This article describes the development and clin. evaluation of two-site immunometric assays for ferritin, TSH, and **tumor necrosis factor**-.alpha. (TNF-.alpha.) using time-resolved **fluorescent** measurement with **streptavidin** -europium (STAV-Eu3+) as label. The liq. phase **antibodies** were labeled with amidocaproylbiotin-N-hydroxysuccinimide ester. All three assays were based on microtiter plate technol. and could be completed within a working day (incubation times less than 4 h). The ferritin assay was compared with luminescent and enzyme-labeled assays using identical components. The TSH assay was compared with a com. immunoluminometric assay whereas the TNF assay was unable to be compared with another method, only with stds. from an independent source. The performance data was excellent with lower detection limits for TSH from <0.003 mU/L and for TNF-.alpha. under 10 ng/L. Intra-assay precision was acceptable within the range of interest with TSH <4% (0.2-50 mU/L), TNF-.alpha. <15% (70-8000 ng/L), and ferritin <8% (10-500 g/L). Interassay precision was <6% for TSH, <16% for TNF-.alpha., and <8.5% for ferritin. All assays were performed using com. available components and proved suitable for routine use.

L27 ANSWER 48 OF 68 CAPLUS COPYRIGHT 1999 ACS

1993:555533 Document No. 119:155533 Preparation of monosubstituted tetrahalopyridines and disubstituted trihalopyridines photochemically grafted at the 4-position to other molecules. Baillarge, Michele; Meziane Cherif, Djalal; Braun, Jacques; Le Goffic, Francois; Francois, Le Goffic (Vegatec S.a.r.L., Fr.). Fr. Demande FR 2676732 A1 19921127, 25 pp. (French). CODEN: FRXXBL. APPLICATION: FR 91-6200 19910523.

AB 4-Azido-2,3,5,6-tetrafluoropyridine (I) and 4-azido-3,5-dichloro-2,6-difluoropyridine are photochem. reacted with a variety of mols., e.g. with polyethylene, polypropylene, latex, polysaccharides, **proteins**, lipids, nucleic acids, cells, etc. The halopyridine may have a nucleophile at the 2-position. The products are useful as supports in peptide and oligonucleotide synthesis, immunoassays, biol., biotechnol. (biocatalysts), etc. (no data). PVDF membranes were immersed in a methanolic soln. of I, dried, irradiated 15 min, and washed with MeOH until the wash soln. absorption at 254 nm dropped to 0. The membranes were then incubated with a soln. of biotin hexamethylene diamine to make membranes for **affinity** purifn. of **streptavidin**.

L27 ANSWER 49 OF 68 CAPLUS COPYRIGHT 1999 ACS

1992:546706 Document No. 117:146706 Cell-free receptor binding tests, their production and use. Lauffer, Leander; Zettlmeissl, Gerd; Oquendo, Patricia (Behringwerke A.-G., Germany). Eur. Pat. Appl. EP 488170 A1 19920603, 25 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE. (German). CODEN: EPXXDW. APPLICATION: EP 91-120187 19911126. PRIORITY: DE 90-4037837 19901128.

AB The binding behavior of cell membrane-localized receptors with a labeled ligand is detd. in cell-free binding tests in which the receptor is provided as a recombinant fusion **protein** with a carrier mol. which can be specifically bound to a solid phase. Thus, the binding properties of a human **interleukin** 4 (IL-4) receptor were studied

by use of the fusion product of the extracellular portion of the human IL-4 receptor with the Fc portion of the heavy chain of a human IgG1 mol. The fusion **protein** was immobilized on an ELISA plate coated with an **affinity**-purified rabbit antiserum to the CH2 domain of human IgG. The ligand for the test was biotinylated IL-4; bound ligand was detected with **streptavidin**-peroxidase.

L27 ANSWER 50 OF 68 MEDLINE DUPLICATE 12
92355624 Document Number: 92355624. Purification of the murine **interleukin** 3 receptor. Mui A L; Kay R J; Humphries R K; Krystal G. (Terry Fox Laboratory, British Columbia Cancer Research Centre, Vancouver, Canada.)JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Aug 15) 267 (23) 16523-30. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB In this report we describe the purification of the murine **interleukin** 3 receptor (mIL-3R) to apparent homogeneity using a two-step procedure involving biotinylated mIL-3 (B-mIL-3) and **affinity** binding to immobilized antiphosphotyrosine and **streptavidin** agarose (SA). Purification was monitored using an assay for detergent solubilized-mIL-3Rs that utilized unglycosylated 125I-mIL-3 and concanavalin A (ConA)-Sepharose beads. The final material consisted of a 140-kDa tyrosine and serine phosphorylated **protein** that was greater than 98% pure as assessed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of either [35S]methionine-labeled, silver-stained, or radioiodinated preparations. Characterization of the purified receptor revealed that it migrated identically under reducing and nonreducing conditions in SDS gels, possessed 10 kDa of N-linked carbohydrate, and was cleaved upon storage at 4 degrees C to a 70-kDa form. These properties suggested that the purified mIL-3R was identical to that identified by cross-linking studies. The KD of the purified receptor was 1-5 nM, similar to estimates obtained using intact normal mouse bone marrow cells and mIL-3-dependent cell lines. The two-step purification procedure also isolated a 120-kDa serine phosphorylated but nontyrosine phosphorylated mIL-3R species. Apart from phosphorylation differences, the 140- and 120-kDa species were apparently identical, yielding, after alkaline phosphatase treatment, the same molecular mass on SDS gels and similar chymotryptic peptide maps. Amino acid sequences and composition data obtained from the more abundant and more stable serine phosphorylated 120-kDa mIL-3R, further purified by SDS-polyacrylamide gel electrophoresis, suggested that the purified mIL-3R may be identical to the predicted sequence of the recently isolated cDNA clone AIC2A. This was further suggested by comparing chymotryptic maps of the 120-kDa mIL-3R with the Aic2A **protein** and using **antibodies** corresponding to the amino and carboxyl termini of the AIC2A cDNA product. However, the Aic2A **protein**, when expressed on the surface of COS or 3T3 cells or following detergent solubilization and partial purification with biotinylated mIL-3 and SA, displayed a substantially lower **affinity** for mIL-3.

L27 ANSWER 51 OF 68 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
92281724 EMBASE Document No.: 1992281724. Cytotoxicity of **streptavidin**-blocked biotinyl-ricin is retrieved by in vitro immunotargeting via biotinyl monoclonal **antibody**. Schechter B.; Arnon R.; Wilchek M.. Department of Chemical Immunology, Weizmann Institute of Science,Rehovot 76100, Israel. Cancer Research 52/16 (4448-4452) 1992.
ISSN: 0008-5472. CODEN: CNREA8. Pub. Country: United States. Language: English. Summary Language: English.

AB The **streptavidin**-biotin system has been used to immunotarget whole ricin to tumor cells in a system that overcomes ricin-nonspecific

cytotoxicity. Biotin was linked to ricin via a disulfide-containing reagent, sulfosuccinimidyl-2-(biotinamido)ethyl-1,3'-dithiopropionate. The product, biotinyl-S,S-ricin (b-ricin), retained most of its in vitro cytotoxic activity against human epidermoid carcinoma (KB) cells. Complexing b-ricin to **streptavidin** resulted in >99% loss of its cellular toxicity which is associated with loss of cell-binding activity. The **streptavidin**-b-ricin complex could, however, be targeted to KB cells via the biotinylated monoclonal **antibody** 108 which is specific to the epidermal **growth factor** receptor overexpressed on KB cells. The complex did not regain its activity if the specific **antibody** was not biotinylated or if the biotinylated **antibody** was of a different specificity. **Streptavidin** is thus used to block b-ricin, presumably due to a steric restraint of the **streptavidin** on the ricin B- chain, and to bridge it to biotinyl **antibody** recognizing the target cell. Avidin could not replace **streptavidin** in this system since a complex between b-ricin and avidin retained a major part (60%) of ricin cytotoxic activity. This is attributed to the nonspecific binding of avidin to cells in vitro, including the KB cells. It is suggested that b-ricin is blocked by both **streptavidin** and avidin, but once the complex gains access to the cell surface, its cytotoxic activity is specifically retrieved.

L27 ANSWER 52 OF 68 MEDLINE DUPLICATE 13

92246874 Document Number: 92246874. The application of a novel biotinylated **affinity** label for the detection of a cathepsin B-like precursor produced by breast-tumour cells in culture. Cullen B M; Halliday I M; Kay G; Nelson J; Walker B. (Division of Biochemistry, School of Biology and Biochemistry, Queen's University, Belfast, Northern Ireland, U.K.)BIOCHEMICAL JOURNAL, (1992 Apr 15) 283 (Pt 2) 461-5. Journal code: 9YO.

ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

AB In this report we demonstrate how the recently developed biotinylated **affinity** label biotinyl-Phe-Ala-diazomethane (Bio-Phe-Ala-CHN2) [Cullen, McGinty, Walker, Nelson, Halliday, Bailie & Kay (1990) Biochem. Soc. Trans. 18, 315-316; Walker, Cullen, Kay, Halliday, McGinty & Nelson (1992) Biochem. J. 283, 449-453] can be used for the detection of a precursor form of a cathepsin B-like enzyme produced by breast-tumour cells in culture. Thus the cell lines MDA-MB-436, ZR-75-1 and T47-D produce a soluble **protein** that can be allowed to react with the biotinylated **affinity** label to yield an SDS-resistant complex; this can be revealed with a **streptavidin**/alkaline phosphatase label after PAGE and Western blotting. This **protein** (molecular mass 47 kDa) can also be detected by immunoblotting using sheep anti-(cathepsin B) **antibodies** in conjunction with a donkey anti-sheep IgG label. None of the cell lines studied produced any mature cathepsin B-like activity, as gauged by the lack of turnover of the fluorogenic substrate benzyloxycarbonyl-Arg-Arg-4-methylcoumarin-7-ylamide (Cbz-Arg-Arg-NH-Mec). However, treatment of medium samples with pepsin resulted in the generation of such activity. When the pepsin-catalysed activation step was analysed by SDS/PAGE, the **protein** of 47 kDa was completely converted into two species of very similar molecular masses of 30.5 kDa and 29 kDa. Both these **proteins** can incorporate the biotinylated probe and, in common with the 47 kD species, they can be detected with the **streptavidin**/alkaline phosphatase label and immunoblotting. We propose that the 47 kD form is the pepsin-activable proform of these lower-molecular-mass species. The release of the proform from the oestrogen-receptor (ER)-positive breast-tumour cell lines ZR-75-1

and T47-D is stimulated 5-10-fold when these cells are grown in medium containing epidermal **growth factor** (EGF) at a concentration of 10 ng/ml. In contrast, there is no modulation in the amount of proform released by the ER-negative cell line MDA-MB-436, over a range of EGF concentrations from 0 to 100 ng/ml.

L27 ANSWER 53 OF 68 CAPLUS COPYRIGHT 1999 ACS
1992:422207 Document No. 117:22207 The application of a novel biotinylated **affinity** label for the detection of a cathepsin B-like precursor produced by breast-tumor cells in culture. Cullen, Breda M.; Halliday, Isla M.; Kay, Gillian; Nelson, John; Walker, Brian (Sch. Biol. Biochem., Queen's Univ., Belfast, BT9 7BL, UK). Biochem. J., 283(2), 461-5 (English) 1992. CODEN: BIJOAK. ISSN: 0306-3275.
AB In this report it is demonstrated how the recently developed biotinylated **affinity** label biotinyl-Phe-Ala-diazomethane (Bio-Phe-Ala-CHN2) can be used for the detection of a precursor form of a cathepsin B-like enzyme produced by breast-tumor cells in culture. Thus the cell lines MDA-MB-436, ZR-75-1 and T47-D produce a sol. **protein** that can be allowed to react with the biotinylated **affinity** label to yield an SDS-resistant complex; this can be revealed with a **streptavidin** /alk. phosphatase label after PAGE and Western blotting. This **protein** (mol. mass 47 kDa) can also be detected by immunoblotting using sheep anti-(cathepsin B) **antibodies** in conjunction with a donkey anti-sheep IgG label. None of the cell lines studied produced any mature cathepsin B-like activity, as gauged by the lack of turnover of the fluorogenic substrate benzyloxycarbonyl-Arg-Arg-4-methylcoumarin-7-ylamide (Cbz-Arg-Arg-NH-Mec). However, treatment of medium samples with pepsin resulted in the generation of such activity. When the pepsin-catalyzed activation step was analyzed by SDS/PAGE, the **protein** of 47 kDa was completely converted into two species of very similar mol. masses of 30.5 kDa and 29 kDa. Both these **proteins** can incorporate the biotinylated probe and, in common with the 47 kD species, they can be detected with the **streptavidin**/alk. phosphatase label and immunoblotting. It is proposed that the 47 kD form is the pepsin-activable proform of these lower-mol.-mass species. The release of the proform from the estrogen-receptor (ER)-pos. breast-tumor cell lines ZR-75-1 and T47-D is stimulated 5-10 fold when these cells are grown in medium contg. epidermal **growth factor** (EGF) at a concn. of 10 ng/mL. In contrast, there is no modulation in the amt. of proform released by the ER-neg. cell line MDA-MB-436, over a range of EGF concn. from 0 to 100 ng/mL.

L27 ANSWER 54 OF 68 MEDLINE
93160723 Document Number: 93160723. Measurement of recombinant **interferon** levels by high performance immunoaffinity chromatography in body fluids of cancer patients on **interferon** therapy. Phillips T M. (Immunochemistry Laboratory, George Washington University Medical Center, Washington, D.C. 20037.)BIOMEDICAL CHROMATOGRAPHY, (1992 Nov-Dec) 6 (6) 287-90. Journal code: BIM. ISSN: 0269-3879. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The technique of high performance immunoaffinity chromatography was used to measure the levels of recombinant **interferon** in chronic lymphocytic leukaemia patients enrolled in a phase II recombinant **interferon** clinical trial. The technique employed a short high pressure chromatography column packed with minute glass beads which had monoclonal **antibody**, directed against recombinant alpha **interferon**, immobilized to their surface. This system was used to

measure **interferon** levels in a variety of different human body fluids. A good correlation was found when **interferon** levels, detected by chromatographic separation, were compared to levels obtained by a conventional radioimmunoassay.

L27 ANSWER 55 OF 68 CAPLUS COPYRIGHT 1999 ACS
1991:225190 Document No. 114:225190 **Cytokine** fusion **proteins** for use in imaging, separations, determinations, and therapy. Svrluga, Richard C.; Waters, Cory A. (Seragen, Inc., USA). PCT Int. Appl. WO 9101004 A1 19910124, 33 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 90-US3828 19900706. PRIORITY: US 89-376656 19890706.

AB **Cytokines** which bind to members of the hematopoietin receptor superfamily (i.e. **interleukins** 2, 3, 4, or 6; erythropoietin; and prolactin) are fused via their N-terminus to another chem. entity such as an enzyme, **streptavidin**, or ricin. These fusion **proteins** have differential **affinity** for the high-, medium-, and low-**affinity** forms of their receptors. This was demonstrated with a diphtheria toxin-**interleukin**-2 fusion **protein** produced by Escherichia coli by observing displacement of labeled **interleukin**-2 from cells lines displaying only 1 of the 3 types of receptor.

L27 ANSWER 56 OF 68 MEDLINE DUPLICATE 14
91177923 Document Number: 91177923. Heavy and light chain variable region sequences and **antibody** properties of anti-phosphotyrosine **antibodies** reveal both common and distinct features. Ruff-Jamison S; Campos-Gonzalez R; Glenney J R Jr. (Lucille P. Markey Cancer Center, Department of Biochemistry, University of Kentucky College of Medicine, Lexington 40536-0093..)JOURNAL OF BIOLOGICAL CHEMISTRY, (1991 Apr 5) 266 (10) 6607-13. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Phosphotyrosine and similar analogs have been used to elicit **antibodies** that have found widespread use in the study of cellular tyrosine phosphorylation. In order to better understand the anti-phosphotyrosine immune response and to elucidate the details of the specific association between a tyrosine phosphate and an **antibody** combining site, we have undertaken a detailed comparison of **antibody** stability, specificity, apparent **affinity**, and primary structure for eight different anti-phosphotyrosine **antibodies** derived from immunizations with three different antigens. Two of these, 2G8 and 1G2, were derived from an immunization using azobenzylphosphonate conjugated to carrier, and five others, Py2, Py20, Py42, Py54, and Py69, were the products of an immunization with phosphotyrosine conjugated to carrier. Each of these **anti-hapten antibodies** was an IgG. One **antibody**, 129, an IgM, was the result of an immunization with a mixture of tyrosine-phosphorylated **proteins** which had been purified from **growth factor** treated cells. We found that **antibody** binding was significantly inhibited by millimolar levels of divalent cations or high concentrations of monovalent salt, with the exception of the **antibody** 129 where binding was significantly enhanced by both. Under optimal conditions, the highest apparent **affinities** for phosphotyrosine were observed for **antibodies** Py69 and Py20 (10(-6)-10(-7) M) and the lowest for 129 and 1G2 (10(-3)-10(-4). The heavy and light chain variable regions of seven of these **antibodies** were cloned and sequenced and a predominant anti-phosphotyrosine response was observed. The light chains of these **antibodies** could be assigned to one of two major VK

groups, VK10 and VK19, with sequence identity between the different light chains of each class ranging from 65 to 100% at the amino acid level. Similar sequence identity was found among the heavy chain sequences (89-98% identity at the amino acid level) with the exception of one **antibody**, 2G8, which was only distantly related to the others (61-64% amino acid identity). These heavy chains belong to the same heavy chain family, J558. Two of the **antibodies**, Py20 and Py69, were clearly derived from the same progenitor cell since both share a highly unusual apparent V-D-D-JH organization. However, a significant level of somatic mutation has occurred between the two **antibodies** resulting in subtle changes in their apparent **affinity** and specificity.

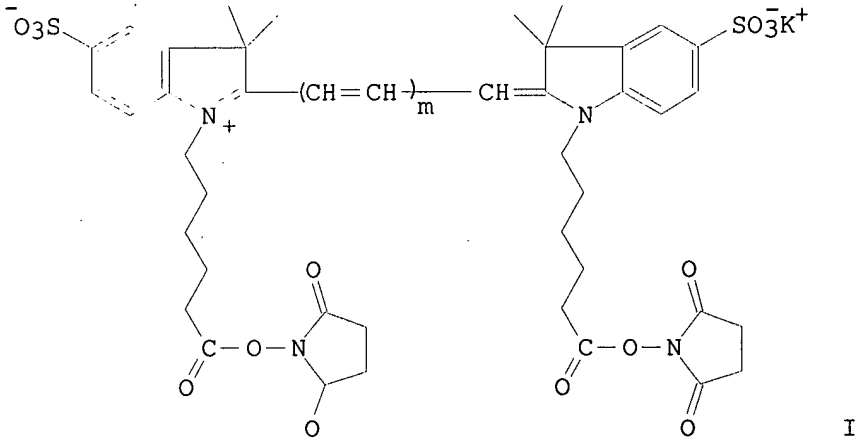
L27 ANSWER 57 OF 68 MEDLINE DUPLICATE 15
92135067 Document Number: 92135067. AIC2A is a component of the purified high **affinity** mouse IL-3 receptor: temperature-dependent modulation of AIC2A structure. Schreurs J; Hung P; May W S; Arai K; Miyajima A. (Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304..)INTERNATIONAL IMMUNOLOGY, (1991 Dec) 3 (12) 1231-42. Journal code: AY5. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.
AB IL-3, a potent hemopoietic **growth factor**, interacts with distinct classes of receptor, one of high **affinity** and the other of low **affinity**. The gene for a 115 kDa, low **affinity** IL-3 binding **protein** (AIC2A) was recently cloned. Ligand **affinity** purification was used to show that the AIC2A gene product participates in the formation of a high **affinity** IL-3 receptor (IL-3R). Cells were incubated with biotin-IL-3 at 4 degrees C and IL-3 bound to the low **affinity** site was removed by washing, cells were detergent extracted, and then **streptavidin** - agarose was used to purify **proteins** bound to biotin-IL-3. A 115 kDa phosphotyrosine (Ptyr)-containing **protein** was specifically purified and its identity as AIC2A was shown in Western assays using polyclonal anti-AIC2A **antibodies**. A brief temperature shift of the intact, biotin-IL-3-treated cells from 4 to 37 degrees C, prior to receptor purification, results in structural and compositional changes in the IL-3R, including: (i) a 10-20 kDa increase in the apparent Mr of both the AIC2A and the Ptyr antigens, and (ii) the association of a serine/threonine kinase. These observations indicate that in its native environment, the low **affinity** IL-3 binding **protein**, AIC2A, participates to form the high **affinity** IL-3R and is a substrate for a tyrosine kinase. Moreover, a ligand-induced, temperature-regulatable structural change in the IL-3R may be of importance in the transduction of information through the receptor, as suggested by the enhanced association of the IL-3R with a serine/threonine kinase.

L27 ANSWER 58 OF 68 MEDLINE
92113296 Document Number: 92113296. A sensitive ELISA for measuring recombinant human **interleukin**-3 in human plasma or serum. Papoian R; Duffy F; Sanner M; Wilt E. (Department of Biopharmaceutics, Sandoz Pharma, Ltd., Basel, Switzerland..)JOURNAL OF IMMUNOLOGICAL METHODS, (1991 Dec 15) 145 (1-2) 161-5. Journal code: IFE. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.
AB We describe a sensitive ELISA for the measurement, of the recombinant human **cytokine**, **interleukin**-3 (IL-3), in human plasma or serum samples. The assay design uses two different anti-IL-3 monoclonal

antibodies, giving a two-site assay configuration. The assay incorporates the use of alkaline phosphatase conjugated to **streptavidin** and a biotinylated anti-IL-3 monoclonal **antibody** to amplify the resultant signal. This ELISA can measure both glycosylated and non-glycosylated IL-3. The limits of quantification, as determined by precision profiles and quality control samples prepared in 100% human plasma, are 20 pg/ml and 30 pg/ml for non-glycosylated and glycosylated IL-3, respectively.

L27 ANSWER 59 OF 68 CAPLUS COPYRIGHT 1999 ACS
1991:58491 Document No. 114:58491 Method of labeling a component of an aqueous fluid with a water-soluble luminescent dye. Waggoner, Alan S. (Carnegie-Mellon University, USA). Ger. Offen. DE 3912046 A1 19900315, 25 pp. (German). CODEN: GWXXBX. APPLICATION: DE 89-3912046 19890412. PRIORITY: US 88-240756 19880902.

GI



I

AB A component (**antibody**, hormone, nucleic acid, virus, cell, drug, etc.) in an aq. liq. is labeled by reacting it with a luminescent cyanine, merocyanine, or styryl dye bearing rl sulfonic acid or sulfonate group which improves the water soly. of the dye and diminishes **fluorescence** quenching. The dye also bears substituents which react with amino, aldehyde, SH, or OH groups of **proteins** or other components to be labeled. Thus, sheep .gamma.-globulin in 0.1M carbonate buffer was incubated with a 10-fold molar excess of sulfoindodicarbocyanine ester I (m = 2) for 10 min and sepd. from noncovalently bound I by gel permeation chromatog. on Sephadex G50. The extent of labeling was greater at pH 9.4 than at pH <9; the quantum yield at pH 9.4 was 0.17. The method is used in spectrochem. anal.

L27 ANSWER 60 OF 68 MEDLINE DUPLICATE 16
91006305 Document Number: 91006305. Characterization of the **interleukin** 5-reactive splenic B cell population. Rolink A G; Thalmann P; Kikuchi Y; Erdei A. (Basel Institute for Immunology, Switzerland..)EUROPEAN JOURNAL OF IMMUNOLOGY, (1990 Sep) 20 (9) 1949-56. Journal code: EN5. ISSN: 0014-2980. Pub. country: GERMANY: Germany,

Federal Republic of. Language: English.

AB The characteristics of the **interleukin** (IL) 5-reactive splenic B cell population of C57BL/6 nu/nu mice, with respect to IL 5/IL2 reactivity, cell surface phenotype, VH gene family usage, autoreactivity and the structure of the IL5 receptor (IL5R), were analyzed. It was found that 2%-4% of splenic B cells express relatively high levels of IL 5R as determined by the binding of the anti-IL 5R monoclonal **antibody** R52.120. Over 90% of the splenic B cells that mature to IgM secretion upon activation with IL5 are comprised in this small subpopulation of B cells. Moreover, the vast majority of splenic B cells that mature to IgM-secreting cells when activated by IL2 also reside in this IL5R+B cell population. The cell surface phenotype of the IL5R+ splenic B cells is IgM+, B220+, Ly-1- and IL2R p55-. Upon activation with IL5 this cell surface phenotype changes, in that a vast majority of the B cells then express the p55 chain of the IL2R, whereas the level of IL5R decreases. VH gene family usage in the IL5-activated splenic B cells was analyzed by in situ hybridization. VH gene family usage was found to be random and not different from the VH genes expressed in LPS-activated B cells. Hybridoma collections from IL5-activated splenic B cells and LPS-activated B cells were screened and compared for the production of autoantibodies and **antibodies** directed against the haptens (4-hydroxy-3-iodo-5-nitrophenyl)acetyl (NIP) and 2,4,6-trinitrophenyl (TNP). In both collections high, but not significantly different frequencies of autoantibody-(32% IL5, 31.4% LPS) and of **anti-hapten antibody** (27.8% IL5, 18.6% LPS)-producing hybridomas were found. The structure of the IL5R on IL5-activated B cells was analyzed by 125I-labeled IL5 binding and cross-linking. About 100 high-**affinity** (10(-11) M) and 1000 low-**affinity** (10(-9) M) IL5-**binding sites** are present on IL5-activated splenic B cells, and both high- and low-**affinity** IL5R are similar to those expressed on the IL5-dependent B13 cell line. Cross-linking of 125I-labeled IL5 to the receptors on IL5-activated B cells revealed one major IL5-binding **protein** of 45-50 kDa molecular mass and another minor binding **protein** of 130-140 kDa. The same IL5-binding **proteins** are present on the IL5-dependent B13 cell line.(ABSTRACT TRUNCATED AT 400 WORDS)

L27 ANSWER 61 OF 68 MEDLINE

91189757 Document Number: 91189757. Epidermal **growth factor** and transferrin receptor expression in human embryonic and fetal epidermal cells. Zambruno G; Girolomoni G; Manca V; Segre A; Giannetti A. (Clinica Dermatologica, Modena, Italy..)ARCHIVES OF DERMATOLOGICAL RESEARCH, (1990) 282 (8) 544-8. Journal code: 6X7. ISSN: 0340-3696. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Epidermal **growth factor** receptors (EGFR) and transferrin receptors (TFR) are known to be involved in cell proliferation and to be expressed in normal human epidermis. To date little is known about EGFR and TRF expression in human skin during embryonic and fetal development. In the present work, we studied skin specimens from 30 aborted embryos and fetuses ranging from 7 to 31 weeks estimated gestational age. Monoclonal **antibodies** to EGFR and TFR were applied on frozen skin sections using an amplification biotin-**streptavidin**-fluorescein technique. TFR was faintly expressed on epidermal basal cells throughout embryonic and fetal development, as it is in adult epidermis. Up to week 12, EGFR was uniformly expressed on cells of the basal, intermediate and periderm cell layers. From the midfetal

period onwards, the suprabasal cell layers showed a decreased staining compared with the basal layer. During the third trimester the cornified cell layer was completely negative. The hair germ and heir peg cells were positive. Later, the outer root sheath and hair bulb remained labelled, with less staining of the hair cone. The sebaceous and eccrine sweat glands were also labelled. These results suggest that in embryonic and fetal epidermis, TFR expression is not correlated with cellular proliferation, whereas EGFR appear to be associated with proliferating and undifferentiated cells.

L27 ANSWER 62 OF 68 MEDLINE DUPLICATE 17
90356151 Document Number: 90356151. Preparation of **affinity**-purified, biotinylated tetanus toxin, and characterization and localization of cell surface **binding sites** on nerve **growth factor**-treated PC12 cells. Fujita K; Guroff G; Yavin E; Goping G; Orenberg R; Lazarovici P. (Section on Growth Factors, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892.)NEUROCHEMICAL RESEARCH, (1990 Apr) 15 (4) 373-83. Journal code: NX9. ISSN: 0364-3190. Pub. country: United States. Language: English.

AB Biotinylated derivatives of tetanus toxin were prepared and isolated by chromatofocusing and ganglioside-**affinity** chromatography. Biotinylation was monitored by the appearance of a 210,00 dalton complex upon SDS-polyacrylamide gel electrophoresis in the presence of avidin, and by selective binding to an avidin-Sepharose gel. At molar biotin:toxin ratios from 1:1 to 20:1 only biotinylated derivatives with low toxicity were obtained; these derivatives, however, retained 60-80% of their specific binding **affinity** for brain synaptosomes. A biotinylated tetanus toxin derivative purified by ganglioside-**affinity** chromatography was used to identify and localize tetanus toxin **binding sites** on PC12 cells. Electron microscopic analysis with **streptavidin**-gold revealed very low levels of tetanus toxin **binding sites** on the surface of untreated cells, and the appearance of such **binding sites** during the second week of nerve **growth factor**-induced differentiation. Examination of micrographs of the differentiated cells indicated that the tetanus toxin **binding sites** sites are concentrated on the neurites, with relatively few appearing on the cell bodies. Cognate studies using 125I-labeled, **affinity**-purified tetanus toxin revealed an increase in PC12 binding capacity from about 0.07 nmol/mg **protein** in untreated cells to 0.8 nmoles/mg **protein** in cells treated for 14 days with nerve **growth factor**. Cells treated in suspension for 2-3 weeks with nerve **growth factor** do not express tetanus toxin **binding sites**; upon plating, these cells required one week for the appearance of **binding sites**, although neurites grew much more rapidly from these "primed" cells. The high binding capacity of these tetanus toxin sites, as well as their sensitivity to neuraminidase, is indicative of a polysialoganglioside structure. The advantages of biotinylated tetanus toxin derivatives are discussed and the significance of nerve **growth factor**-differentiated PC12 cells grown as monolayers as a model for the study of the development, localization, and function of neuraminidase-sensitive tetanus toxin **binding sites** is presented.

L27 ANSWER 63 OF 68 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
89285883 EMBASE Document No.: 1989285883. **Interleukin**-3 stimulates the tyrosine phosphorylation of the 140-kilodalton **interleukin**-3

receptor. Sorensen P.; Mui A.L.-F.; Krystal G.. Terry Fox Laboratory, British Columbia Cancer Research Centre, Vancouver, BC V5Z 1L3, Canada. Journal of Biological Chemistry 264/32 (19253-19258) 1989. ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.

AB Murine **interleukin**-3 (mIL-3) stimulates the rapid and transient tyrosine phosphorylation of a number of **proteins** in mIL-3-dependent B6SUtA1 cells. Two of these **proteins**, p68 and p140, are maximally phosphorylated at tyrosine residues within 2 min of addition of mIL-3. Because 125I-mIL-3 can be cross-linked to both 70- and 140-kDa **proteins** on intact B6SUtA1 cells, we investigated whether the tyrosine phosphorylated p68 and p140 were these two mIL-3 receptor **proteins**. Addition of antiphosphotyrosine **antibodies** (.alpha.PTyr Abs) to cell lysates from B6SUtA1 cells, to which 125I-mIL-3 had been disuccinimidyl suberate-cross-linked, resulted in the immunoprecipitation of 125I-mIL-3 complexed to both 70- and 140-kDa **proteins**. To determine if the observed immunoprecipitation pattern was due to the direct interaction of .alpha.-PTyr Abs with these two mIL-3 receptor **proteins** that were associated with the receptor **proteins**, cell lysates were treated with 2% sodium docecyl sulfate, 5% 2-mercaptoethanol, and boiled for 1 min. After removal of sodium docecyl sulfate and 2-mercaptoethanol, .alpha.PTyr Abs immunoprecipitated 125I-mIL-3 cross-linked to only the 140-kDa **protein**. To confirm this finding, 32P-labeled B6SUtA1 cells were treated with biotinylated or fluoresceinated mIL-3. Addition of immobilized **streptavidin** of antifluorescein **antibodies**, respectively, to cell lysates from these cells resulted in the enrichment of only a 140-kDa tyrosine phosphorylated **protein**. Taken together, these results strongly suggest that only the 140-kDa receptor **protein** is tyrosine phosphorylated upon mIL-3 binding.

L27 ANSWER 64 OF 68 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD

AN 88-353928 [49] WPIDS

AB WO 8809344 A UPAB: 970926

A single chain multi-functional biosynthetic **protein** expressed from a single gene derived by recombinant DNA techniques is claimed, the **protein** comprising (a) a biosynthetic **antibody binding site** capable of binding to a preselected antigenic determinant and comprising at least one **protein** domain, the amino acid sequence of the domain being homologous to at least a portion of the sequence of a variable region of an immunoglobulin molecule capable of binding the preselected antigenic determine and, peptide bonded to this (b) a polypeptide selected from effector **proteins** having a conformation suitable biological activity in mammals, amino acid sequences capable of sequestering an ion and amino acid sequences capable of selective binding to a solid support.

The effector **protein** may be e.g. an enzyme, toxin, receptor, **binding site**, **growth factor**, **lymphokine**, **cytokine** anti-metabolite.

The sequence capable of sequestering an ion may be e.g. calmodulin or metallothionein. The sequence capable of binding to a solid support is e.g. **streptavidin** or a fragment of **protein** A.

USE/ADVANTAGE - The **proteins** may be used for e.g. specific binding assays, **affinity** purificn., biocatalysts, drug targeting, imaging and immunological treatment of oncogenid and infectious diseases. They offer fewer cleavage sites to circulating proteolytic enzymes and have improved stability. They reach their target tissue rapidly and are cleared quickly from the body. They also have reduced

immunogenicity. Their design facilitates coupling to other moieties in drug targeting and imaging applications.
Dwg.0/15

ABEQ US 5091513 A UPAB: 930923
Single polypeptide (PP) chain comprises (A) a PP linker defining a single and complete site for binding a preselected antigen (PA) and spanning the distance between (B) the C-terminus of 1 PP domain having an amino acid (AA) sequence comprising a heavy chain variable region and (C) the N-terminus of other PP domain having an AA sequence comprising a light chain variable region. (B) and/or (C) comprise a recombinant PP comprising
(a) a set of complementary determining regions (CDR) AA sequences defining
a recognition site for the PA, (b) a set of framework regions (FR) AA sequences linked to the CDR sequences with the linked sets (a) and (b) defining a hybrid immunoglobulin variable region binding domain immunologically reactive with the PA and (c) a 3rd AA sequence, peptide bonded to the C- and N-termini of the **binding site** and consisting of a single PP chain having a conformation conferring biological activity to the 3rd sequence under the same conditions as allowing binding of the site for binding to the PA. The biological activity is independent of the **binding site**.
The 3rd AA sequence is pref. peptide bonded to the C-terminus of the **binding site** and is esp. a PP immunologically reactive with an antigen. A radioactive atom is esp. bound to the PP chain.
ADVANTAGE - A PP defines a structure capable of selective antigen recognition and preferential antigen binding.

ABEQ US 5132405 A UPAB: 930923
Single polypeptide chain comprises two polypeptide domains connected by a polypeptide linker spanning the distance between the C- and N-termini of the domains, defining a single and complete **binding site** for a preselected antigen. Aminoacid sequence of one domain comprises a heavy chain variable region and the other a light chain variable region. At least one of the domains comprises a recombinant peptide, contg. CDR amino acids which define a recognition site for the antigen and a set of FR aminoacid sequences linked to the CDR sequences. The two sets together define a hybrid chimaeric imunoglobulin variable region binding domain. A third set of aminoacids conferring biological activity to allow binding to
the preselected antigen is also present.
USE/ADVANTAGE - **Binding sites** are used in specific binding assays, **affinity** purificn., biocatalysis, drug targetting, imaging, immunological treatment of oncogenic and infectious diseases.
0/7

ABEQ US 5258498 A UPAB: 931220
Polypeptide linking sequence comprises at least 10 hydrophilic aminoacid units (not contg. -Cys-) linked by peptide bonds, having a flexible, unstructured configuration, free from secondary structure in aq. soln., with a chain length sufficient to bridge two biologically active synthetic
polypeptide domains at specific sites, from the C-terminus of one domain to the N-terminus of the other.
The polypeptide (Gly)4Ser(Gly)4Ser(Gly)4Ser is a typical example. These polypeptide gps. are produced by condensn. of lower peptides and/or aminoacids, or by recombinant DNA technology.
USE - The prods. facilitate specific binding assays, **affinity** chromatography biocatalysts, imaging, drug targetting and the immunological treatment of oncogenic and infectious diseases, etc.
Dwg.0/15

ABEQ EP 318554 B UPAB: 950518

A single chain multi-functional biosynthetic **protein** expressed from a single gene derived by recombinant DNA techniques, said **protein** comprising: a biosynthetic **antibody binding site** capable of binding to a preselected antigenic determinant and comprising an amino acid sequence homologous with the sequence of a variable region of an immunoglobulin molecule capable of binding said preselected antigenic determinant, a first biofunctional domain comprising a polypeptide selected from the group consisting of effector **proteins** having a conformation suitable for biological activity in mammals, amino acid sequences capable of sequestering an ion, and amino acid sequences capable of selective bonding to a solid support, and a first polypeptide linker disposed between said **binding site** and said first biofunctional domain, wherein said polypeptide linker comprises plural, hydrophilic, peptide-bonded amino acids and which defines a polypeptide which connects the C-terminal end of said **binding site** and the N-terminal end of said first biofunctional domain or the N-terminal end of said **binding site** and the C-terminal end of said first biofunctional domain, whereupon said binding **protein** assumes a conformation suitable for binding and said first biofunctional domain assumes a conformation suitable for biological activity, sequestering an ion, or selectively binding a solid support.

Dwg.0/15

ABEQ US 5476786 A UPAB: 960205

A new host cell harbours and is capable of expressing a DNA encoding a single chain polypeptide, said single chain polypeptide comprising

two polypeptide domains connected by a polypeptide linker spanning the distance between the C-terminus of one domain to the N-terminus of the other and defining a single and complete site for binding a preselected antigen, wherein the amino acid sequence of one of said polypeptide domains comprises a heavy chain variable region, and the amino acid sequence of the other of said polypeptide domains comprises a light chain variable region, wherein at least one of said polypeptide domains comprises,

a recombinant polypeptide comprising,

a set of CDR amino acid sequences together defining a recognition site for said preselected antigen,

a set of FR amino acid sequences linked to said set of CDR sequences,

said linked sets of CDR and FR amino acid sequences together defining a hybrid immunoglobulin variable region binding domain which is immunologically reactive with said preselected antigen and

a third amino acid sequence, peptide bonded to the N- or C-terminus of said site for binding, said third amino acid sequence comprising a single polypeptide chain having a conformation which confers biological activity to said third sequence under the same conditions that allow binding to said preselected antigen, said biological activity being independent of said site for binding.

Dwg.0/7

ABEQ US 5482858 A UPAB: 960227

A host cell harbouring and capable of expressing a DNA encoding a biosynthetic single chain polypeptide, said single chain polypeptide comprises;

a linking sequence connecting first and second non-naturally peptide-bonded, biologically active polypeptide domains to form a single polypeptide chain comprising at least two biologically active domains, connected by the linking sequence, the linking sequence comprising

hydrophilic, peptide-bonded amino acids comprising at least 10 amino acid residues, the linking sequence being cysteine-free, having a flexible unstructured polypeptide configuration essentially free of secondary structure in aqueous solution, having a plurality of glycine or serine residues and defining a polypeptide of a length sufficient to span the distance between the C-terminal end of the first domain and the N-terminal end of the second domain.
Dwg.0/15

L27 ANSWER 65 OF 68 MEDLINE
89035439 Document Number: 89035439. Construction of antigen-specific suppressor T cell hybridomas from spleen cells of mice primed for the persistent IgE **antibody** formation. Iwata M; Ishizaka K. (Subdepartment of Immunology, Johns Hopkins University School of Medicine, Baltimore, MD 21239.)JOURNAL OF IMMUNOLOGY, (1988 Nov 15) 141 (10) 3270-7. Journal code: IFB. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB Attempts were made to generate Ag-specific suppressor T cells from Ag-primed spleen cells by using glycosylation inhibiting factor (GIF). BDF1 mice were primed with alum-absorbed OVA and their spleen cells were stimulated with OVA. Ag-activated T cells were then propagated in IL-2-containing conditioned medium. Incubation of the T cells with OVA-pulsed syngeneic macrophages resulted in the formation of IgE-potentiating factor and glycosylation-enhancing factor that has **affinity** for OVA, i.e., OVA-specific glycosylation-enhancing factor. However, if the same Ag-activated splenic T cells were propagated in the IL-2-containing medium in the presence of GIF T cells obtained in the cultures formed IgE-suppressive factors and OVA-specific GIF on antigenic stimulation. Thus we constructed T cell hybridomas from the Ag-activated T cells propagated by IL-2 in the presence of GIF. A representative hybridoma, 71B4, formed OVA-specific GIF on incubation with OVA-pulsed macrophages of BDF1 mice or C57B1/6 mice. However, if the same hybridoma cells were incubated with OVA alone or with OVA-pulsed macrophages of H-2k or H-2d strains, they produced GIF that had no **affinity** for OVA. The OVA-specific GIF bound to OVA-Sepharose but did not bind to BSA-Sepharose or KLH Sepharose. Intravenous injections of the OVA-specific GIF from the hybridoma suppressed the IgE and IgG1 anti-DNP **antibody** response of BDF1 mice to DNP-OVA, but failed to suppress the **anti-hapten antibody** responses of the strain to DNP-keyhole limpet hemocyanin, indicating that the factors suppressed the **antibody** response in a carrier-specific manner. However, the same OVA-specific GIF failed to suppress the **anti-hapten antibody** response of DBA/1 mice to DNP-OVA, suggesting that the immunosuppressive effects of the factors is MHC restricted.

L27 ANSWER 66 OF 68 MEDLINE
88140371 Document Number: 88140371. Establishment of a highly sensitive enzyme-linked immunosorbent assay for **interleukin**-1 alpha employing a fluorogenic substrate. Mukaida N; Kasahara T; Ko Y; Kawai T. (Department of Clinical Pathology, Jichi Medical School, Tochigi-ken, Japan..)JOURNAL OF IMMUNOLOGICAL METHODS, (1988 Feb 24) 107 (1) 41-6. Journal code: IFE. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.
AB We have previously established a non-competitive solid-phase enzyme-linked immunosorbent assay (ELISA) specific for **interleukin**-1 alpha

(IL-1 alpha) using a combination of **polyclonal antibody** as the immobilized **antibody**, biotinylated monoclonal **antibody** as the second **antibody** and avidin-peroxidase. The level of detection of that ELISA was 200-500 pg/ml. In order to improve its sensitivity, we have used **streptavidin** -beta-D-galactosidase and the fluorogenic substance 4-methylumbelliferyl-D- galactopyranoside as enzyme substrate. With this system IL-1 alpha could be detected at concentrations as low as 10-50 pg/ml, which was about 10-20 times more sensitive than conventional mouse thymocyte co-stimulator assays. Furthermore, the assay system was specific for IL-1 alpha in that neither IL-1 beta nor **interleukin**-2 (IL-2) interfered.

L27 ANSWER 67 OF 68 MEDLINE

87110712 Document Number: 87110712. Carrier-specific suppression of **antibody** responses by antigen-specific glycosylation-inhibiting factors. Jardieu P; Akasaki M; Ishizaka K. JOURNAL OF IMMUNOLOGY, (1987 Mar 1) 138 (5) 1494-501. Journal code: IFB. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB We previously established an ovalbumin (OA)-specific T cell clone from spleen cells of BDF1 mice, which had been treated by i.v. injections of OA, and constructed antigen-specific T cell hybridomas from the T cell clone. One of the hybridomas constitutively released glycosylation-inhibiting factor (GIF) which lacked **affinity** for OA, and was called non-specific GIF. Incubation of the same hybridoma cells with OA-pulsed syngeneic macrophages or OA-pulsed B lymphoblastoid cells of BALB/c origin resulted in the formation of GIF molecules that had **affinity** for OA but not for bovine serum albumin or keyhole limpet hemocyanin. Both the OA-specific GIF and nonspecific GIF bound to monoclonal anti-lipocortin and possessed I-Jb determinants. The OA-specific GIF consisted of two species of molecules, of m.w. 80,000 and 30,000 to 40,000, respectively, whereas the nonspecific GIF from unstimulated cells had an m.w. of 15,000. Intravenous injections of OA-specific GIF or nonspecific GIF into BDF1 mice suppressed both the IgE and IgG1 **anti-hapten antibody** responses of the animals to dinitrophenyl derivatives of OA (DNP-OA), but OA-specific GIF was much more effective than nonspecific GIF in suppressing the **antibody** responses. When the same preparations of GIF were injected into DNP-KHL-primed mice, OA-specific GIF and nonspecific GIF were comparable in suppressing the anti-DNP **antibody** response. In contrast to the 40,000 m.w. species of OA-specific GIF, the 80,000 m.w. OA-specific GIF had carrier-specific suppressive effects. The similarities of antigen-specific GIF to antigen-specific TsF suggest that the phospholipase-inhibiting activity of the molecules may be involved in the immunosuppressive effects of some antigen-specific TsF.

L27 ANSWER 68 OF 68 CAPLUS COPYRIGHT 1999 ACS

1984:188438 Document No. 100:188438 Modified labeled nucleotides and polynucleotides and methods of utilizing and detecting them. Engelhardt, Dean; Rabbani, Elazar; Kline, Stanley; Stavrianopoulos, Jannis G.; Kirtikar, Dollie (Enzo Biochem, Inc., USA). Eur. Pat. Appl. EP 97373 A2 19840104, 140 pp. DESIGNATED STATES: R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 83-106112 19830622. PRIORITY: US 82-391440 19820623.

AB Nucleotides, polynucleotides, and DNA were chem. modified or labeled with chem. moieties which were readily detectable. These chem. moieties included carbohydrates and sugars, electron dense substances, magnetic substances, enzymes, coenzymes, hormones, radioactive substances, metals,

fluorescent substances, antigens, or **antibodies**. These chem. modified nucleotides were used for: (1) stimulating or inducing cells to produce **lymphokines**, cytokinins, and **interferon** ; (2) testing resistance of bacteria to antibiotics; (3) diagnosing genetic disorders, e.g., .beta.-thalassemia; (4) diagnosing tumors; (5) diagnosing bacteria, virus, or fungus infection; and (6) karyotyping chromosomes.

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L30 434 FILE BIOSIS
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L31 232 FILE EMBASE
L32 2 FILE WPIDS

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L52 ANSWER 1 OF 8 MEDLINE DUPLICATE 1
1998189774 Document Number: 98189774. In vivo IL-4 responses to anti-IgD **antibody** are MHC class II dependent and beta 2-microglobulin independent and develop normally in the absence of IL-4 priming of T cells. **Morris S C**; Coffman R L; **Finkelman F D**.
(Department of Medicine, University of Cincinnati College of Medicine, OH 45267, USA.)JOURNAL OF IMMUNOLOGY, (1998 Apr 1) 160 (7) 3299-304.
Journal code: IFB. ISSN: 0022-1767. Pub. country: United States.
Language:
English.
AB A crucial role for CD1-responsive, MHC class II-unrestricted T cells in the generation of T cell IL-4 responses is suggested by the: 1) requirement for IL-4 to prime in vitro IL-4 responses by naive CD4+ T cells; 2) ability of TCR cross-linking to induce CD1-responsive T cells, but not conventional naive T cells, to produce IL-4; 3) failure of anti-IgD Ab to induce an IL-4-dependent IgE response in beta 2-microglobulin-deficient mice, which lack CD1; and 4) reported ability of MHC class II-deficient mice to make IgE responses to anti-IgD Ab. In contrast, the Ag specificity of **cytokine** and Ab responses in anti-IgD-injected mice and the normal IgE responses made by anti-IgD-treated CD1-deficient mice are difficult to reconcile with this view. We now find that the failure of beta 2-microglobulin-deficient mice to make an IgE response to anti-IgD Ab is caused by their rapid degradation of anti-IgD; sustained anti-IgD treatment induces them to make relatively normal IL-4 and IgE responses. Furthermore, in our study, MHC class II-deficient mice make little or no IL-4 or IgE responses to anti-IgD Ab and beta 2-microglobulin-deficient mice make large in vivo IL-4 responses to anti-CD3 mAb. Finally, although IL-4 priming of T cells for IL-4 production is Stat6 dependent, Stat6-deficient mice make normal IL-4 responses to anti-IgD. Thus, CD1-responsive T cells and other beta 2-microglobulin-dependent T cells are not required to prime conventional CD4+ T cells to make IL-4 responses to anti-IgD in vivo; in fact, the large IL-4 response made in this system does not require IL-4 priming.

L52 ANSWER 2 OF 8 MEDLINE DUPLICATE 2
1998150970 Document Number: 98150970. IL-13, IL-4Ralpha, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite Nippostrongylus brasiliensis. Urban J F Jr; Noben-Trauth N; Donaldson D D; Madden K B; **Morris S C**; Collins M; **Finkelman F D**.
(United States Department of Agriculture, Beltsville, Maryland 20705, USA.
)IMMUNITY, (1998 Feb) 8 (2) 255-64. Journal code: CCF. ISSN: 1074-7613.
Pub. country: United States. Language: English.
AB Although IL-4 induces expulsion of the gastrointestinal nematode parasite,

Nippostrongylus brasiliensis, from immunodeficient mice, this parasite is expelled normally by IL-4-deficient mice. This apparent paradox is explained by observations that IL-4 receptor alpha chain (IL-4Ralpha)-deficient mice and Stat6-deficient mice fail to expel N. brasiliensis, and a specific antagonist for IL-13, another activator of Stat6 through IL-4Ralpha, prevents worm expulsion. Thus, N. brasiliensis expulsion requires signaling via IL-4Ralpha and Stat6, and IL-13 may be more important than IL-4 as an inducer of the Stat6 signaling that leads to worm expulsion. Additional observations made in the course of these experiments demonstrate that Stat6 signaling is not required for IL-4 enhancement of IgG1 production and actually inhibits IL-4-induction of mucosal mastocytosis.

L52 ANSWER 3 OF 8 MEDLINE DUPLICATE 3
97272122 Document Number: 97272122. Effects of blocking B7-1 and B7-2 interactions during a type 2 in vivo immune response. Greenwald R J; Lu P;
Halvorson M J; Zhou X; Chen S; Madden K B; Perrin P J; **Morris S C**; **Finkelman F D**; Peach R; Linsley P S; Urban J F Jr; Gause W C. (Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA.)JOURNAL OF IMMUNOLOGY, (1997 May 1) 158 (9) 4088-96. Journal code: IFB. ISSN: 0022-1767. Pub. country: United
States. Language: English.
AB The costimulatory signal provided to T cells through CD28/CTLA-4 interactions is required for in vivo Th cell effector function associated with **cytokine** production. However, it is uncertain whether the two well-characterized ligands for these molecules, B7-1 and B7-2, differentially influence the consequent development of a type 1 or a type 2 primary response. We have examined the in vivo effects of blocking B7-1 and/or B7-2 ligand interactions on the type 2 mucosal immune response that
follows oral infection of mice with the nematode parasite, Heligmosomoides
polygyrus. Administration of the combination of anti-B7-1 and anti-B7-2 Abs inhibited H. polygyrus-induced increases in serum IgG1 and IgE levels,
the expansion of mesenteric lymph node (MLN) germinal centers, in situ CD4+ T cell expansion, elevated blood eosinophils, and increased intestinal mucosal mast cells. Similarly, both Abs blocked MLN and Peyer's
patch **cytokine** gene expression and elevations in MLN T cell-derived IL-4 **protein** secretion. However, in the same experiments, administration of either anti-B7-1 or anti-B7-2 Abs alone had
little effect on any of these parameters. T cell and B cell activation was
also blocked by the combination of anti-B7-2 and a B7-1-specific mutant Y100F CTLA-4Ig construct. These results suggest that to the extent that anti-B7-1 and anti-B7-2 mAbs block B7 interactions, either B7-1 or B7-2 ligand interactions can provide the required costimulatory signals that lead to T cell effector function during a type 2 in vivo immune response.

L52 ANSWER 4 OF 8 MEDLINE
96322725 Document Number: 96322725. Dendritic cells can present antigen in vivo in a tolerogenic or immunogenic fashion. **Finkelman F D**; Lees A; Birnbaum R; Gause W C; **Morris S C**. (Department of Medicine, University of Cincinnati College of Medicine, OH 45267, USA.)JOURNAL OF IMMUNOLOGY, (1996 Aug 15) 157 (4) 1406-14. Journal code: IFB.
ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Dendritic cells (DC) are unmatched among APCs in their ability to bind, process, and present Ag. Presentation by such potent APCs, if always immunogenic and never tolerogenic, might stimulate pathogenic autoimmune responses. To determine whether Ag presentation by DC can induce tolerance, mice were injected with a rat IgG2b anti-splenic DC mAb, 33D1, and challenged 13 to 28 days later with a stimulatory rat IgG2b mAb. Injection of mice with 1 ng/100 micrograms of 33D1 rarely induced an anti-rat IgG2b Ab response and, in most mice, induced rat IgG2b-specific T cell and B cell tolerance. Tolerant mice had decreased ability to secrete Ab and make both type 1 and type 2 **cytokine** mRNA and **protein** in response to immunization with rat IgG2b. 33D1 was 100- to 1000-fold more potent as a tolerogen than an isotype-matched control rat IgG2b mAb. Injecting mice with aggregated 33D1, 33D1 plus anti-IgD mAb, or 33D1 plus IL-1 induced an IgG1 anti-rat IgG2b Ab response rather than tolerance. IL-1 injected 3 days after 33D1 still induced an Ab response rather than tolerance. Not all anti-DC mAbs are tolerogenic. Injection of a DC-specific hamster anti-CD11c mAb (N418) stimulates an IgG anti-hamster response, and injection of 33D1 plus N418 stimulates both anti-hamster and anti-rat IgG2b responses. These observations indicate that DCs can present Ag in either a tolerogenic or stimulatory manner and suggest that inflammatory stimuli can convert an otherwise tolerogenic signal to a stimulatory signal.

L52 ANSWER 5 OF 8 MEDLINE
97086728 Document Number: 97086728. H. polygyrus: B7-independence of the secondary type 2 response. Gause W C; Lu P; Zhou X D; Chen S J; Madden K B; **Morris S C**; Linsley P S; **Finkelman F D**; Urban J F. (Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814, USA.)EXPERIMENTAL PARASITOLOGY, (1996 Nov) 84 (2) 264-73. Journal code: EQP. ISSN: 0014-4894. Pub. country: United States. Language: English.

AB The gastrointestinal nematode parasite, Heligmosomoides polygyrus, has been used extensively in experimental studies of host immunity. The pronounced type 2 primary immune response to H. polygyrus is associated with elevated CD4+, TCR-alpha/beta + T cell IL-4 production and elevated serum IgE levels that are blocked by inhibiting CD28/ CTLA4-B7 interactions following in vivo administration of the chimeric fusion **protein**, CTLA4Ig. In the present study, we have examined the in vivo effects of blocking CTLA4Ig ligands on the secondary type 2 mucosal host protective immune response to this parasite. Our results show that although CD4+, TCR-alpha/beta + cells remain the primary source of elevated IL-4 during the secondary response, the protective immune response and the effector cell activity associated with it is B7-independent as CTLA4Ig administration at the time of challenge does not block (1) elevations in T cell IL-4 gene expression or **protein** secretion; (2) elevations in serum IgE levels, mucosal mastocytosis, or eosinophilia; or (3) host protection, as measured by adult worm burden and fecundity. These findings suggest that memory T helper cells do not require CD28-B7 interactions for their activation to effector cells that can mediate a host protective type 2 immune response.

L52 ANSWER 6 OF 8 MEDLINE DUPLICATE 4
94194138 Document Number: 94194138. Induction of B cell and T cell tolerance in vivo by anti-CD23 mAb. **Morris S C**; Lees A; Holmes J M; Jeffries R D; **Finkelman F D**. (Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814..)JOURNAL

OF IMMUNOLOGY, (1994 Apr 15) 152 (8) 3768-76. Journal code: IFB. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB T cell tolerance can be induced by B cell presentation of Ags to naive T cells. To further characterize this mechanism of T cell tolerance induction, we have investigated the effects of injecting mice with an intact rat IgG2a Ab, which binds to the B cell low-affinity Fc epsilon receptor (CD23), on the responsiveness of B cells and T cells to rat IgG2a. Our observations indicate that 1) intravenous, subcutaneous, or intraperitoneal injection of this Ab induces antigen-specific B cell and T cell tolerance; 2) both forms of tolerance are induced more completely by injection of rat IgG2a anti-CD23 mAb than by injection of an equal dose of a control rat IgG2a Ig; and 3) reduced responsiveness to Ag is seen as early as 1 to 3 days after anti-CD23 mAb injection and reaches maximum levels by 7 days after injection. Although tolerance induced by the injection of soluble **proteins** has been reported to be characterized by reduced production of IL-2 and IFN-gamma, but normal production of IL-4, injection of mice with rat IgG2a anti-mouse CD23 mAb greatly decreases the IL-4 response to a rat IgG2a immunogen that normally induces a large IL-4 response.

L52 ANSWER 7 OF 8 MEDLINE DUPLICATE 5

94132598 Document Number: 94132598. Effects of IL-12 on in vivo **cytokine** gene expression and Ig isotype selection. **Morris S C**; Madden K B; Adamovicz J J; Gause W C; Hubbard B R; Gately M K; **Finkelman F D**. (Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814..)JOURNAL OF IMMUNOLOGY, (1994 Feb 1) 152 (3) 1047-56. Journal code: IFB. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The effects of murine rIL-12 on **cytokine** gene expression and Ig secretion were studied in vivo. In untreated mice IL-12 enhanced IFN-gamma and IL-10 gene expression and **protein** secretion, reduced base line IL-3 and IL-4 gene expression, and increased serum IgG2a concentration. In mice that had been injected with goat anti-mouse IgD **antibody** (G alpha M delta) to induce increases in IL-3, IL-4, and IL-10 gene expression and serum IgE, IgG1, IgG2a, and IgG3 concentrations, the simultaneous injection of IL-12 enhanced IFN-gamma and IL-10 gene expression and suppressed IL-3 and IL-4 gene expression and serum IgG and IgE responses. Anti-IFN-gamma mAb neutralized most, but not all, IFN-gamma produced by mice treated with G alpha M delta and IL-12. Anti-IFN-gamma mAb enhanced IL-3 and IL-4 gene expression, did not affect IL-10 or IFN-gamma gene expression, and increased serum IgG1, IgG2a, and IgG3 levels, but had relatively little effect on serum IgE in these mice. In contrast to its effects in G alpha M delta-treated mice, IL-12 failed to inhibit the IgE response to G alpha M epsilon **antibody**, which stimulates mIgE+ B cells to secrete IgE. These observations demonstrate that: 1) IL-12 may limit its own effects by inducing the production of a **cytokine** (IL-10) that down-regulates both IL-12 production and IL-12-induced IFN-gamma production; 2) IL-12 inhibits the production of at least one **cytokine**, IL-3, that is not generally regarded to be strictly Th1- or Th2-associated; 3) IL-12 inhibits switching to IgE secretion to a greater extent than it inhibits switching to other Ig isotypes; and 4) the in vivo effects of IL-12 are, to a large extent, IFN-gamma-dependent.

L52 ANSWER 8 OF 8 MEDLINE DUPLICATE 6
93329102 Document Number: 93329102. Anti-**cytokine** **antibodies** as carrier **proteins**. Prolongation of in vivo effects of exogenous **cytokines** by injection of **cytokine** -anti-**cytokine** **antibody** complexes. **Finkelman F** **D**; Madden K B; **Morris S C**; Holmes J M; Boiani N; Katona I M; Maliszewski C R. (Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.)JOURNAL OF IMMUNOLOGY, (1993 Aug 1) 151 (3) 1235-44. Journal code: IFB. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Anti-**cytokine** **antibodies** that block interactions between **cytokines** and **cytokine** receptors have been used to inhibit endogenous **cytokine** function. However, injection of mice with mixtures of IL-4 and either of two neutralizing anti-IL-4 mAb, at a **cytokine**/anti-**cytokine** mAb molar ratio of approximately 2:1, enhances and prolongs in vivo IL-4 activity, as measured by induction of increased spleen cell Ia expression. Although splenocyte Ia expression returns to baseline two days after mice are injected with free IL-4, soluble IL-4-anti-IL-4 mAb complexes still induce several-fold increases in Ia expression 3 days after injection. Complexes that contain as little as 400 ng of IL-4 have considerable in vivo stimulatory activity, and a maximal effect on splenocyte Ia expression is induced by injection of 2 micrograms of complexed IL-4. The stimulatory effect of IL-4-containing complexes on splenocyte Ia expression can be blocked by increasing the ratio of anti-IL-4 mAb to IL-4, by injection of anti-IL-4R mAb, and by in vivo aggregation of the complexes. Complexes of IL-4 with a non-neutralizing anti-IL-4 mAb do not have increased IL-4 agonist activity in vivo. These observations are most consistent with the possibility that neutralizing anti-IL-4 mAb act as carrier **proteins** that increase the in vivo half-life of IL-4 by preventing its excretion, and possibly, by preventing modification of its active site. The enhanced agonist effect of IL-4-anti-IL-4 mAb complexes is not unique; complexes of IL-3 with a neutralizing anti-IL-3 mAb have a greatly increased ability, compared with free IL-3, to stimulate mucosal mastocytosis, and complexes of IL-7 with a neutralizing anti-IL-7 mAb have a greatly increased ability, compared with free IL-7 or IL-7 complexed with a non-neutralizing anti-IL-7 mAb, to stimulate an increase in pre-B cell number. These observations suggest that complexes of **cytokines** and neutralizing anti-**cytokine** mAb may provide a generally useful way to increase the magnitude and duration of **cytokine** effects in vivo.

=> s (interleukin? or interferon? or chemokine? or lymphotoxin? or lymphokine? or growth factor or colony stimulating factor or tumor necrosis factor? or l1 or cytokine or target analyte or macromolecule or protein) and (body fluid or saliva or blood or extracellular fluid)

L53 230935 FILE MEDLINE
L54 161020 FILE CAPLUS
L55 216046 FILE BIOSIS
L56 150570 FILE EMBASE
'CN' IS NOT A VALID FIELD CODE
L57 8201 FILE WPIDS

TOTAL FOR ALL FILES
L58 766772 (INTERLEUKIN? OR INTERFERON? OR CHEMOKINE? OR LYMPHOTOXIN? OR

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                LYMPHOKINE? OR GROWTH FACTOR OR COLONY STIMULATING FACTOR OR
                TUMOR NECROSIS FACTOR? OR L1 OR CYTOKINE OR TARGET ANALYTE OR
                MACROMOLECULE OR PROTEIN) AND (BODY FLUID OR SALIVA OR BLOOD
OR
                EXTRACELLULAR FLUID)

=> s l58 and (radioisotope or affinity label or enzymatic label or
fluorescent label)

L59        5091 FILE MEDLINE
L60         314 FILE CAPLUS
L61         137 FILE BIOSIS
L62       10950 FILE EMBASE
L63          69 FILE WPIDS

TOTAL FOR ALL FILES
L64       16561 L58 AND (RADIOISOTOPE OR AFFINITY LABEL OR ENZYMATIC LABEL OR
                FLUORESCENT LABEL)

=> s (streptavidin or anti biotin or "anti-biotin" or "anti-hapten" or ani
igg or l2) and l64

L65          34 FILE MEDLINE
L66           6 FILE CAPLUS
L67           4 FILE BIOSIS
L68          10 FILE EMBASE
'CN' IS NOT A VALID FIELD CODE
L69           1 FILE WPIDS

TOTAL FOR ALL FILES
L70          55 (STREPTAVIDIN OR ANTI BIOTIN OR "ANTI-BIOTIN" OR "ANTI-HAPTEN"
                OR ANI IGG OR L2) AND L64

=> s l70 not 126

L71          34 FILE MEDLINE
L72           5 FILE CAPLUS
L73           4 FILE BIOSIS
L74           9 FILE EMBASE
L75           1 FILE WPIDS

TOTAL FOR ALL FILES
L76          53 L70 NOT L26

=> s l76 not 151

L77          34 FILE MEDLINE
L78           5 FILE CAPLUS
L79           4 FILE BIOSIS
L80           9 FILE EMBASE
L81           1 FILE WPIDS

TOTAL FOR ALL FILES
L82          53 L76 NOT L51

=> dup rem l82

PROCESSING COMPLETED FOR L82
L83          42 DUP REM L82 (11 DUPLICATES REMOVED)

=> d cbib abs 1-42;dis his
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L83 ANSWER 1 OF 42 MEDLINE
1999110624 Document Number: 99110624. Retention of biologic activity of human epidermal **growth factor** following conjugation to a **blood**-brain barrier drug delivery vector via an extended poly(ethylene glycol) linker. Deguchi Y; Kurihara A; Pardridge W M. (Department of Medicine, UCLA School of Medicine, Los Angeles, California 90095-1682, USA.)BIOCONJUGATE CHEMISTRY, (1999 Jan-Feb) 10 (1) 32-7. Journal code: A1T. ISSN: 1043-1802. Pub. country: United States.
Language:
English.

AB Human brain gliomas overexpress the receptor for epidermal **growth factor** (EGF), and radiolabeled EGF is a potential peptide radiopharmaceutical for imaging human brain tumors, should this peptide be made transportable through the **blood**-brain barrier (BBB) in vivo. Peptide drug delivery to the brain may be facilitated by conjugating peptide radiopharmaceuticals to BBB drug delivery vectors such as the OX26 monoclonal antibody (MAb), which undergoes receptor-mediated transcytosis through the BBB via the brain capillary endothelial transferrin receptor. EGF was biotinylated with NHS-XX-biotin, where NHS = N-hydroxysuccinimide and -XX- = bis (aminohexanoyl) spacer arm. The [125I]EGF-XX-biotin rapidly bound to C6 rat glioma cells transfected with the human EGF receptor. However, no binding to the C6 EGF receptor was detected when the [125I]EGF-XX-biotin was bound to a conjugate of **streptavidin** (SA) and the OX26 MAb. An alternative linker strategy using poly(ethylene glycol) (PEG) of 3400 Da molecular mass (PEG3400) was evaluated, wherein EGF was monobiotinylated with NHS-PEG3400-biotin. Attachment of the [125I]EGF-PEG3400-biotin to the OX26/SA conjugate did not impair binding of the construct to the EGF receptor in C6 glioma cells. The length of the -PEG- spacer arm and the -XX- spacer arm was >200 atoms and 14 atoms, respectively. These studies demonstrate that the use of the extended PEG linker releases steric hindrance of MAb transport vectors on binding of EGF to its cognate receptor on glioma cells. Attachment of EGF peptide radiopharmaceuticals to BBB drug delivery systems such as the OX26 MAb using extended PEG linkers allows for retention of the bifunctionality of the conjugate with binding to both EGF and transferrin receptors.

L83 ANSWER 2 OF 42 MEDLINE DUPLICATE 1
1998425440 Document Number: 98425440. Assessment of bovine platelet life span with biotinylation and flow cytometry. Baker L C; Kameneva M V; Watach M J; Litwak P; Wagner W R. (Department of Bioengineering, Pittsburgh, Pennsylvania, USA.)ARTIFICIAL ORGANS, (1998 Sep) 22 (9) 799-803. Journal code: 8ZK. ISSN: 0160-564X. Pub. country: United States.
Language: English.

AB Reduced platelet life span is associated with the implantation of a variety of cardiovascular devices and may be used as a gauge of device biocompatibility. In the bovine model, platelet life span has previously been assessed with **radioisotope** labeling of removed platelets followed by reinjection and periodic gamma counting of **blood** samples. We report here the use of **protein**-reactive biotin (sulfo-N-hydroxysuccinimido [NHS]-biotin) as an alternative to **radioisotope** techniques whereby reinjected biotinylated platelets are subsequently detected in **blood** samples using phycoerythrin-**streptavidin** and flow cytometric techniques. Platelet life span

was quantified in a normal calf (4.9 days) and in a calf prior to (6.1 days) and following (3.1 days) implantation of a Nimbus Axial Flow Pump ventricular assist device. The assessment of bovine platelet life span with biotinylation and flow cytometry avoids the technical, regulatory, and safety considerations associated with **radioisotope** usage and appears readily amenable to application in cardiovascular device testing.

L83 ANSWER 3 OF 42 MEDLINE
1999023510 Document Number: 99023510. Recombinant IA-2 expressed in E. coli can be used for the routine detection of autoantibodies in Type-I diabetes. Morgenthaler N G; Lobner K; Morgenthaler U Y; Christie M R; Seissler J; Scherbaum W A. (Department of Medicine III, University of Leipzig, Germany.. morgenthaler@brahms.de). HORMONE AND METABOLIC RESEARCH, (1998 Sep) 30 (9) 559-64. Journal code: GBD. ISSN: 0018-5043. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB We have investigated the possibility of measuring autoantibodies to IA-2 (IA-2A) using recombinant **protein** expressed in E. coli in a new radioassay. The intracellular part of IA-2 (IA-2ic) was expressed in E. coli as a biotinylated fusion **protein** and affinity-purified on a **streptavidin** column. The average yield of IA-2ic was about 1 mg purified **protein** from one litre of culture medium with E. coli. We could demonstrate the immunological activity of this material by blocking the autoantibody reactivity to in vitro synthesised IA-2ic. The IA-2ic fusion **protein** was then radiolabelled with 125I, purified by HPLC, and used in an immunoprecipitation assay for the detection of IA-2A. Sera from 46 of 68 (67%) patients with Type-I diabetes were positive by this radioassay, in contrst to only 2 of 50 (4%) patients with autoimmune thyroid disease and 1 of 114 (1 %) controls. There was a correlation between this radioassay and the previously established radioligand assay using synthesized 35S-methionine-labelled IA-2ic in vitro ($r = 0.79$, $p < 0.001$). We conclude that E. coli-derived IA-2 has the correct immunogenic conformation, and can be used for the detection of IA-2A with a similar sensitivity and specificity as the validated radioligand assay. This new assay can facilitate the measurement of IA-2A in routine laboratories where the radioligand assay is inconvenient or not available.

L83 ANSWER 4 OF 42 MEDLINE
97460007 Document Number: 97460007. Drug targeting of a peptide radiopharmaceutical through the primate **blood**-brain barrier in vivo with a monoclonal antibody to the human insulin receptor. Wu D; Yang J; Pardridge W M. (Department of Medicine, UCLA School of Medicine, Los Angeles, California 90095-1682, USA.)JOURNAL OF CLINICAL INVESTIGATION, (1997 Oct 1) 100 (7) 1804-12. Journal code: HS7. ISSN: 0021-9738. Pub. country: United States. Language: English.
AB Peptide radiopharmaceuticals are potential imaging agents for brain disorders, should these agents be enabled to undergo transport through the **blood**-brain barrier (BBB) in vivo. Radiolabeled Abeta1-40 images brain amyloid in tissue sections of Alzheimer's disease autopsy brain, but this peptide radiopharmaceutical cannot be used to image brain amyloid in vivo owing to negligible transport through the BBB. In these studies, 125I-Abeta1-40 was monobiotinylated (bio) and conjugated to a BBB drug delivery and brain targeting system comprised of a complex of the 83-14 monoclonal antibody (mAb) to the human insulin receptor, which is tagged with **streptavidin** (SA). A marked increase in rhesus monkey brain uptake of the 125I-bio-Abeta1-40 was observed after conjugation to the

8314-SA delivery system at 3 h after intravenous injection. In contrast, no measurable brain uptake of 125I-bio-Abeta1-40 was observed in the absence of a BBB drug delivery system. The peptide radiopharmaceutical was degraded in brain with export of the iodide radioactivity, and by 48 h after intravenous injection, 90% of the radioactivity was cleared from the brain. In conclusion, these studies describe a methodology for BBB drug delivery and brain targeting of peptide radiopharmaceuticals that could be used for imaging amyloid or other brain disorders.

L83 ANSWER 5 OF 42 MEDLINE DUPLICATE 2

97403090 Document Number: 97403090. Development of a **streptavidin**-anti-carcinoembryonic antigen antibody, radiolabeled biotin pretargeting method for radioimmunotherapy of colorectal cancer. Reagent development. Karacay H; Sharkey R M; Govindan S V; McBride W J; Goldenberg D M; Hansen H J; Griffiths G L. (Immunomedics, Inc., Morris Plains, New Jersey 07950, USA.)BIOCONJUGATE CHEMISTRY, (1997 Jul-Aug) 8 (4) 585-94. Journal code: A1T. ISSN: 1043-1802. Pub. country: United States. Language: English.

AB With pretargeting, **radioisotope** delivery to tumor is decoupled from the long antibody localization process, and this can increase tumor:**blood** ratios dramatically. Several reagents were prepared for each step of a "two-step" pretargeting method, and their properties were investigated. For pretargeting tumor, **streptavidin**-monoclonal antibody (StAv-mab) conjugates were prepared by cross-linking sulfo-SMCC-derivatized **streptavidin** to a free thiol (SH) group on MN-14 [a high-affinity anti-carcinoembryonic antigen (CEA) mab]. Thiolated mabs were generated either by reaction of 2-iminothiolane (2-IT) with mab lysine residues or by reduction of mab disulfide bonds with (2-mercaptoethyl)amine (MEA). Both procedures gave **protein-protein** conjugates isolated in relatively low yields (20-25%) after preparative size-exclusion (SE) chromatography purification with conservative peak collection. Both StAv-MN-14 conjugates retained their ability to bind to CEA, to an anti-idiotypic antibody to MN-14 (WI2), and to biotin, as demonstrated by SE-HPLC. Two clearing agents, WI2 mab and a biotin-human serum albumin (biotin-HSA) conjugate, were developed to remove excess circulating StAv-MN-14 conjugates in animals. Both clearing **proteins** were also modified with galactose residues, introduced using an activated thioimidate derivative, to produce clearing agents which would clear rapidly and clear primary mab rapidly. At least 14 galactose residues on WI2 were required to reduce **blood** levels to 5.9 +/- 0.7% ID/g in 1 h. Faster **blood** clearance (0.7 +/- 0.2% ID/g) was observed in 1 h using 44 galactose units per WI2. For the delivery of **radioisotope** to tumor, several biotinylated conjugates consisting of biotin, a linker, and a chelate were prepared. Conjugates showed good in vitro and in vivo stability when D-amino acid peptides were used as linkers, biotin-peptide-DOTA-indium-111 had a slightly longer **blood** circulation time (0.09 +/- 0.02% ID/g in 1 h) than biotin-peptide-DTPA-indium-111 (0.05 +/- 0.03% ID/g in 1 h) in nude mice. A longer circulation time with the neutral DOTA complex might allow higher tumor uptake.

L83 ANSWER 6 OF 42 MEDLINE

97255490 Document Number: 97255490. Decreased renal accumulation of biotinylated chimeric monoclonal antibody-neocarzinostatin conjugate after administration of avidin. Otsuji E; Yamaguchi T; Yamamoto K; Matsumura H; Tsuruta H; Yata Y; Nishi H; Okamoto K; Kitamura K; Takahashi T. (First Department of Surgery, Kyoto Prefectural University of Medicine.)JAPANESE

JOURNAL OF CANCER RESEARCH, (1997 Feb) 88 (2) 205-12. Journal code: HBA. ISSN: 0910-5050. Pub. country: Japan. Language: English.

AB Murine monoclonal antibodies (mAbs) such as A7 administered to humans induce a human anti-mouse antibody response. Moreover, because Fab fragments of mAbs are able to penetrate target tumors easily, they may be more suitable than intact mAb to be carriers of anticancer agents such as neocarzinostatin (NCS), which are rapidly inactivated in the **blood** . To address these problems, chimeric A7 Fab fragment-NCS conjugate (chA7Fab-NCS) was produced. However, large amounts of 125I-labeled chA7Fab-NCS accumulate in the kidney and can lead to renal dysfunction. To decrease renal accumulation of chA7Fab-NCS, chA7Fab was biotinylated and administered with a subsequent injection of avidin. Human pancreatic carcinoma-bearing nude mice were injected with 125I-labeled biotinylated chA7Fab-NCS with or without subsequent administration of avidin. The accumulation of 125I-labeled biotinylated chA7Fab-NCS in tissue samples was measured at appropriate time intervals. 125I-labeled biotinylated chA7Fab-NCS was cleared more rapidly from the **blood** and the kidney with the administration of avidin than without it. There was no difference between tumor accumulation in these groups. The tumor/**blood** ratio of radioactivity of 125I-labeled biotinylated chA7Fab-NCS was significantly higher with subsequent administration of avidin than without avidin. The administration of biotinylated chA7Fab-NCS followed by avidin may enhance safety and permit the administration of larger doses of NCS without the subsequent development of renal failure. A larger amount of 125I-labeled biotinylated chA7Fab-NCS was retained in the liver and spleen with the subsequent administration of avidin than without avidin.

L83 ANSWER 7 OF 42 MEDLINE

97235353 Document Number: 97235353. Intravenous avidin chase improved localization of radiolabeled **streptavidin** in intraperitoneal xenograft pretargeted with biotinylated antibody. Zhang M; Sakahara H; Yao Z; Saga T; Nakamoto Y; Sato N; Nakada H; Yamashina I; Konishi J. (Department of Nuclear Medicine, Faculty of Medicine, Kyoto University, Japan.)NUCLEAR MEDICINE AND BIOLOGY, (1997 Jan) 24 (1) 61-4. Journal code: BOO. ISSN: 0969-8051. Pub. country: ENGLAND: United Kingdom. Language: English.

AB In the present study, we examined the effect of avidin administered intravenously (i.v.) on the biodistribution of radiolabeled **streptavidin** in mice bearing intraperitoneal (IP) xenografts pretargeted with biotinylated antibody. Tumors were established in nude mice by IP inoculation of LS180 human colon cancer cells. Monoclonal antibody MLS128, which recognizes Tn antigen on mucin, was biotinylated and injected IP into the IP tumor-bearing mice. Radioiodinated **streptavidin** was administered IP or i.v. 48 h after pretargeting of biotinylated antibody. Avidin was administered i.v. 30 min prior to **streptavidin** injection. The localization of radioiodinated **streptavidin** in the tumor pretargeted with biotinylated antibody was significantly higher than that without pretargeting and that of radioiodinated MLS128 by the one-step method. Avidin administration significantly accelerated the clearance of radioiodinated **streptavidin** in **blood** and other normal tissues and increased the tumor-to-**blood** radioactivity ratio regardless of administration route of **streptavidin**. The i.v. avidin chase improved tumor localization of radiolabeled **streptavidin** in the

IP xenografts pretargeted with biotinylated antibody.

L83 ANSWER 8 OF 42 MEDLINE
96320316 Document Number: 96320316. Biochemical modification of **streptavidin** and avidin: in vitro and in vivo analysis. Rosebrough S F; Hartley D F. (Department of Radiology, University of Rochester Medical Center, New York 14642, USA.)JOURNAL OF NUCLEAR MEDICINE, (1996 Aug) 37 (8) 1380-4. Journal code: JEC. ISSN: 0161-5505. Pub. country: United States. Language: English.
AB The high affinity **streptavidin** (or avidin)/biotin system is being investigated for imaging and radiotherapy procedures. **Streptavidin** (SA) and avidin exhibit markedly different pharmacokinetics, with avidin clearing from the **blood** much faster than SA. To optimize **blood** clearance kinetics, SA and avidin were biochemically modified and analyzed in vitro and in vivo. METHODS: Galactose moieties were covalently attached to promote binding by hepatocyte galactose receptors and hasten SA clearance. To prolong avidin clearance, avidin was deglycosylated and/or neutralized by acetylation of its lysine amino acids. In vitro, the modified **proteins** were analyzed by isoelectric focusing, SDS polyacrylamide electrophoresis and a biotin binding saturation assay. The modified and native **proteins** were radiolabeled with 131I and injected into rabbits for pharmacokinetic, redistribution and imaging analysis. RESULTS: For SA, the resulting increase in **blood** clearance and liver accumulation was correlated to the amount of galactose bound to SA. For avidin, each type of modification increased its circulation time, with the slowest clearance resulting from a combination of deglycosylation and neutralization. CONCLUSION: Biochemical modification of SA and avidin resulted in altered pharmacokinetics compared to the native **proteins**. Modified SA or avidin, when cross-linked with a lesion-specific targeting agent, may be applicable for rapid two-step in vivo imaging techniques.

L83 ANSWER 9 OF 42 MEDLINE
96223497 Document Number: 96223497. Galactose-modified **streptavidin** -GC4 antifibrin monoclonal antibody conjugates: application for two-step thrombus/embolus imaging. Rosebrough S F; Hashmi M. (Department of Radiology, University of Rochester Medical Center, New York, USA.)JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, (1996 Feb) 276 (2) 770-5. Journal code: JP3. ISSN: 0022-3565. Pub. country: United States. Language: English.
AB Diagnostic and therapeutic procedures utilizing the high affinity **streptavidin** (SA)/biotin system are being investigated for in vivo use. We are developing a rapid two-step imaging technique for the diagnosis of deep venous thrombosis and pulmonary embolism. The optimal SA-bound targeting moiety would circulate adequately for sufficient lesion accumulation, but nonbound reagent would clear in a reasonably short time before the injection of radiolabeled biotin. The objective of this study was to cross-link SA and galactose-modified SA to GC4 antifibrin monoclonal antibody and to study the pharmacokinetics and biodistribution of the radiolabeled GC4-SA conjugates after injection into rabbits. A cross-linking method was developed for the synthesis of the GC4-SA conjugates via the addition reaction of sulfhydryl containing SA derivatives with maleimide-GC4. In vivo, radiolabeled trigalactose modified SA-GC4 exhibited a much faster **blood** clearance compared

to mono-galactose modified GC4-SA or GC4-SA containing no galactose.

L83 ANSWER 10 OF 42 MEDLINE
96036057 Document Number: 96036057. Vector-mediated delivery of 125I-labeled
beta-amyloid peptide A beta 1-40 through the **blood**-brain barrier and binding to Alzheimer disease amyloid of the A beta 1-40/vector complex. Saito Y; Buciak J; Yang J; Pardridge W M. (Department of Medicine, School of Medicine, University of California, Los Angeles 90024,
USA.)PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES
OF AMERICA, (1995 Oct 24) 92 (22) 10227-31. Journal code: PV3. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB The brain amyloid of Alzheimer disease (AD) may potentially be imaged in patients with AD by using neuroimaging technology and a radiolabeled form of the 40-residue beta-amyloid peptide A beta 1-40 that is enabled to undergo transport through the brain capillary endothelial wall, which makes up the **blood**-brain barrier (BBB) in vivo. Transport of 125I-labeled A beta 1-40 (125I-A beta 1-40) through the BBB was found to be negligible by experiments with both an intravenous injection technique and an internal carotid artery perfusion method in anesthetized rats. In addition, 125I-A beta 1-40 was rapidly metabolized after either intravenous injection or internal carotid artery perfusion. BBB transport was increased and peripheral metabolism was decreased by conjugation of monobiotinylated 125I-A beta 1-40 to a vector-mediated drug delivery system, which consisted of a conjugate of **streptavidin** (SA) and the OX26 monoclonal antibody to the rat transferrin receptor, which undergoes receptor-mediated transcytosis through the BBB. The brain uptake, expressed as percent of injected dose delivered per gram of brain,
of the 125I,bio-A beta 1-40/SA-OX26 conjugate was 0.15 +/- 0.01, a level that is 2-fold greater than the brain uptake of morphine. The binding of the 125I,bio-A beta 1-40/SA-OX26 conjugate to the amyloid of AD brain was demonstrated by both film and emulsion autoradiography performed on frozen
sections of AD brain. Binding of the 125I,bio-A beta 1-40/SA-OX26 conjugate to the amyloid of AD brain was completely inhibited by high concentrations of unlabeled A beta 1-40. In conclusion, these studies show
that BBB transport and access to amyloid within brain may be achieved by conjugation of A beta 1-40 to a vector-mediated BBB drug delivery system.

L83 ANSWER 11 OF 42 MEDLINE
95238390 Document Number: 95238390. Ubiquitin is conjugated to the cytoskeletal **protein** alpha-spectrin in mature erythrocytes. Corsi D; Galluzzi L; Crinelli R; Magnani M. (Institute of Biological Chemistry G. Fornaini, University of Urbino, Italy.)JOURNAL OF BIOLOGICAL
CHEMISTRY, (1995 Apr 14) 270 (15) 8928-35. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB Ubiquitination of red **blood** cell (RBC) **proteins** was investigated by encapsulation of 125I-ubiquitin into human erythrocytes using a procedure of hypotonic dialysis, isotonic resealing, and reannealing. Incubation (37 degrees C, up to 2 h) of 125I-ubiquitin-loaded
cells resulted in the recovery of 125I-ubiquitin with the cytosolic **proteins** (9.22 +/- 0.4 micrograms/ml RBC) and conjugated to membrane **proteins** (2.18 +/- 0.05 micrograms/ml RBC). This conjugation was time-dependent, and the predominant membrane **protein** band that became labeled showed an apparent molecular mass

of 240 kDa on SDS-polyacrylamide gel electrophoresis (PAGE). Western blotting experiments with three different anti-ubiquitin antibodies revealed that this **protein** is also ubiquitinated in vivo. Cell-free experiments have shown that fraction II (a DEAE-bound **protein** fraction eluted by 0.5 M KCl) prepared from both mature erythrocytes and reticulocytes is able to conjugate ubiquitin to this **protein**. Ubiquitin conjugation was ATP-dependent (Km 0.09 mM), time-dependent, and fraction II-dependent (8 +/- 0.5 pmol of 125I-ubiquitin/h/mg of fraction II). Isolation of the major RBC membrane **protein** that is ubiquitinated was obtained by using biotinylated ubiquitin. Membrane **proteins**, once ubiquitinated with this derivative, were extracted and purified by affinity chromatography on immobilized avidin. The major components retained by the column were two peptides of molecular masses 220 and 240 kDa. Both **proteins** are recognized by a monoclonal anti-spectrin antibody, but only the 240-kDa component is detected by **streptavidin** peroxidase conjugate. That indeed the ubiquitinated membrane **protein** of 240-kDa is alpha-spectrin was confirmed by immunoaffinity chromatography using 125I-ubiquitin and a monoclonal anti-spectrin antibody. Antigen-antibody complexes were purified by **protein** A chromatography and analyzed by SDS-PAGE and autoradiography. Again two bands of 240 and 220 kDa were eluted (alpha- and beta-spectrin), but only one band corresponding to the electrophoretic mobility of alpha-spectrin was detected by autoradiography. Thus, alpha-spectrin is a substrate for the ATP-dependent ubiquitination system, suggesting that the cytoskeleton is covalently modified by ubiquitination both in reticulocytes and mature RBC.

L83 ANSWER 12 OF 42 MEDLINE

95256983 Document Number: 95256983. Improved targeting of radiolabeled **streptavidin** in tumors pretargeted with biotinylated monoclonal antibodies through an avidin chase. Yao Z; Zhang M; Kobayashi H; Sakahara H; Nakada H; Yamashina I; Konishi J. (Department of Nuclear Medicine, Faculty of Medicine, Kyoto University, Japan.)JOURNAL OF NUCLEAR MEDICINE, (1995 May) 36 (5) 837-41. Journal code: JEC. ISSN: 0161-5505. Pub. country: United States. Language: English.

AB Radiolabeled **streptavidin** can be accumulated in tumors pretargeted with biotinylated anti-tumor antibodies. However, circulating biotinylated antibody and endogenous biotin may interfere with the tumor targeting of **streptavidin**. To reduce biotinylated antibody concentration in the **blood**, we injected avidin before **streptavidin** administration. The effects of avidin administration on the biodistribution and tumor targeting of radiolabeled **streptavidin** were examined. METHODS: Biotinylated anti-human colon cancer monoclonal antibody (MAb) MLS128 was injected intravenously into nude mice bearing human colon cancer xenografts for pretargeting. After intraperitoneal injection of avidin, radioiodinated **streptavidin** was administered and its biodistribution and tumor accumulation was investigated. RESULTS: Radioiodinated **streptavidin** specifically localized in the tumor pretargeted with biotinylated antibody. Avidin preadministration accelerated the tumor uptake and **blood** clearance of radioiodinated **streptavidin**. The tumor-to-**blood** radioactivity ratio at 6 and 24 hr after radiolabeled **streptavidin** injection were 1.23 +/- 0.29 and 3.04 +/- 0.86, respectively, in mice with avidin chase (mean +/- s.d., n = 7), and 0.82 +/- 0.17 and 2.29 +/- 0.29, respectively, in those without chase (mean +/- s.d., n = 7). CONCLUSION: Localization of radiolabeled **streptavidin** in tumors pretargeted with biotinylated MAb could be improved by avidin chase. This approach may be useful for tumor radioimmunoimaging and radioimmunotherapy.

L83 ANSWER 13 OF 42 MEDLINE
95263374 Document Number: 95263374. Comparison of the chase effects of avidin, **streptavidin**, neutravidin, and avidin-ferritin on a radiolabeled biotinylated anti-tumor monoclonal antibody. Kobayashi H; Sakahara H; Endo K; Hosono M; Yao Z S; Toyama S; Konishi J. (Department of Radiology and Nuclear Medicine, Faculty of Medicine, Kyoto University.)JAPANESE JOURNAL OF CANCER RESEARCH, (1995 Mar) 86 (3) 310-4. Journal code: HBA. ISSN: 0910-5050. Pub. country: Japan. Language: English.

AB Injection of avidin can decrease the background radioactivity due to a radiolabeled biotinylated monoclonal antibody. We compared the chase effects of avidin, **streptavidin**, neutravidin, and avidin-conjugated ferritin on a radiolabeled antitumor monoclonal antibody in tumor-bearing nude mice. A radioiodine-labeled biotinylated monoclonal antibody (OST7) was administered to athymic mice bearing osteogenic sarcomas. After 24 h, an avidin, **streptavidin**, neutravidin or avidin-conjugated ferritin chaser was intravenously injected into the mice. At 2 h after the chase, the biodistribution of the radiolabeled monoclonal antibody was determined. Clearance from the **blood** was dose-dependently accelerated by avidin and its effect was 10-fold stronger than that of neutravidin or avidin-ferritin. **Streptavidin** did not promote clearance of the biotinylated antibody. Avidin was the most effective chasing agent for improving the biodistribution of the radiolabeled biotinylated monoclonal antibody among the four avidin derivatives tested.

L83 ANSWER 14 OF 42 MEDLINE
95226485 Document Number: 95226485. Recombinant metallothionein-conjugated **streptavidin** labeled with 188Re and 99mTc. Virzi F; Winnard P Jr; Fogarasi M; Sano T; Smith C L; Cantor C R; Rusckowski M; Hnatowich D J. (Department of Nuclear Medicine, University of Massachusetts Medical Center, Worcester 01655, USA.)BIOCONJUGATE CHEMISTRY, (1995 Jan-Feb) 6 (1) 139-44. Journal code: A1T. ISSN: 1043-1802. Pub. country: United States. Language: English.

AB Consideration is now being given to the use of avidin (or **streptavidin**) and biotin for radiotherapy of tumor. Accordingly, the goal of this study was to radiolabel a mouse metallothionein-**streptavidin** fusion **protein** with 188Re and to compare its properties to those of the same fusion **protein** radiolabeled with 99mTc. A recombinant metallothionein-**streptavidin** fusion **protein** was radiolabeled by transchelation with 99mTc- and 188Re-glucoheptonate. Labeling efficiency, which was not optimized for either radionuclide, was approximately 60% for 99mTc and 20% for 188Re. Radiochemical purity was demonstrated by size exclusion HPLC both by nearly quantitative shifts of the 188Re label to higher molecular weight upon the addition of biotinylated antibody and by the absence of a shift with biotinsaturated 188Re-metallothionein-**streptavidin**. Stability of the labels in 37 degrees C serum was evaluated by comparing the HPLC radiochromatograms of serum samples both before and after the addition of biotinylated antibody. The 188Re label behaved like 99mTc in that the same peaks were evident, including one prominent peak due to labeled cysteine. Recoveries during HPLC analysis of serum samples showed that oxidation rates to perrhenate and pertechnetate were identical. However, instability to cysteine challenge was greater for 188Re; for example, the loss of label to cysteine after 24 h under one set of conditions was 41% for 188Re and 22% with 99mTc. Analysis by HPLC of liver and kidney homogenates from mice administered the labeled antibodies were

qualitatively and, in large measure, quantitatively independent of label. Biodistributions at 5 h in normal mice were statistically identical between the two labels in **blood** and in most tissues. In conclusion, **streptavidin** may be radiolabeled with radiorhenium using recombinant mouse metallothionein as a bifunctional chelator, and under one set of labeling conditions at least, 188Re showed similar in vitro and in vivo behavior to that of 99mTc labeled to the same fusion **protein**.

L83 ANSWER 15 OF 42 MEDLINE
95334253 Document Number: 95334253. Influence of endogenous biotin on the biodistribution of labelled biotin derivatives in mice. Rusckowski M; Fogarasi M; Virzi F; Hnatowich D J. (Department of Nuclear Medicine, University of Massachusetts Medical Center, Worcester 01655, USA.)NUCLEAR
MEDICINE COMMUNICATIONS, (1995 Jan) 16 (1) 38-46. Journal code: OB8. ISSN: 0143-3636. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Previously, this laboratory reported that in mice pre-targeted with unlabelled **streptavidin**, the biodistribution of 111In administered on one biotin derivative (EB1) was superior to that of another derivative (DB2). In addition, a Scatchard analysis showed that the affinity constant of 111In-EB1 is lower by seven orders of magnitude from that of 111In-DB2. Therefore, this paper considers the role that endogenous biotin may play in these observations. Both 111In-labelled EB1 and DB2 were bound to **streptavidin** and incubated at 37 degrees C in mouse **blood** with increasing concentrations of d-biotin. As determined by Sephadex G-50 chromatography, only an 8-fold molar excess of
d-biotin relative to labelled **streptavidin** was required to displace 90% of label in the case of EB1, whereas even a 20-fold molar excess provided no detectable displacement of DB2. That this displacement was also occurring in vivo was established in a mouse model bearing an infected thigh: increasing the serum biotin level (by intraperitoneal administration of d-biotin) had no effect on the biodistribution of 111In when administered on DB2; however, the target to non-target ratio decreased in the case of EB1. We have also observed that the biodistribution is no longer favourable when EB1 is administered radiolabelled with 99Tcm. When 111In was substituted with 99Tcm on EB1, chromatography of **blood** samples showed that similar displacement was occurring; however, in this case, the displaced label bound to serum **proteins**.(ABSTRACT TRUNCATED AT 250 WORDS)

L83 ANSWER 16 OF 42 MEDLINE
95118794 Document Number: 95118794. Galactosylated **streptavidin** for improved clearance of biotinylated intact and F(ab')2 fragments of an anti-tumour antibody. Marshall D; Pedley R B; Melton R G; Boden J A; Boden
R; Begent R H. (Department of Clinical Oncology, Royal Free Hospital School of Medicine, London, UK.)BRITISH JOURNAL OF CANCER, (1995 Jan) 71 (1) 18-24. Journal code: AV4. ISSN: 0007-0920. Pub. country: SCOTLAND: United Kingdom. Language: English.
AB Persistence of high levels of radiolabelled antibody in the circulation is
a major limitation of radioimmunotherapy. Biotinylation of the radiolabelled anti-tumour antibody followed by administration of **streptavidin** is known to give much improved tumour to **blood** ratios as the radioantibody is complexed and subsequently cleared via the reticuloendothelial system, although prolonged splenic uptake is a problem. We have investigated the effect on the clearance pattern and tumour localisation of a 125I-labelled biotinylated anti-CEA

antibody (A5B7) after administration of a galactosylated form of **streptavidin** (gal-**streptavidin**) in nude mice bearing a human colon carcinoma xenograft. Fifteen minutes to 1 h after gal-**streptavidin** administration the complexes were cleared via the liver alone (as opposed to liver and spleen after native **streptavidin**). Twenty-four hours after administration of gal-**streptavidin**, the tumour to **blood** ratio for biotinylated A5B7 IgG increased from 2.9 to 13.2 and for biotinylated F(ab')2 fragments an increase from 4.9 to 33.2 was achieved. The reduction in tumour accumulation of F(ab')2 24 h after injection of the clearing agent was less than that seen with intact antibody. Injection of asialofetuin inhibited clearance, confirming that removal of the gal-**streptavidin**-biotinylated antibody complexes from the **blood** was via the asialoglycoprotein receptor on liver hepatocytes. Therefore, galactosylation of the **streptavidin** clearing agent allows rapid removal of radiolabelled biotinylated antibodies via the liver asialoglycoprotein receptor, as opposed to the reticuloendothelial system.

L83 ANSWER 17 OF 42 CAPLUS COPYRIGHT 1999 ACS
1994:442743 Document No. 121:42743 Microparticle formulations containing biodegradable mixed polymers. Fritzsch, Thomas; Heldmann, Dieter; Weitschies, Werner (Schering A.-G., Germany). Ger. Offen. DE 4232755 A1 19940331, 8 pp. (German). CODEN: GWXXBX. APPLICATION: DE 92-4232755 19920926.

AB Microcapsules for diagnostic or therapeutic use are prepd. comprising (1) a wall of biopolymer/synthetic polymer interpolymer, which has site-specific phys. properties or bears functional groups for binding e.g. chelating ligands or their metal complexes, and (2) an optional core consisting of a gas or therapeutic agent. Thus, an autoclaved gelatin soln. was adjusted to pH 5.0 and stirred with Bu cyanoacrylate to produce air-filled dispersible microparticles [55 mol% gelatin, 45 mol% poly(Bu cyanoacrylate)] for use in contrast media for sonog.

L83 ANSWER 18 OF 42 MEDLINE
94228538 Document Number: 94228538. **Streptavidin** distribution in metastatic tumors pretargeted with a biotinylated monoclonal antibody: theoretical and experimental pharmacokinetics. Sung C; van Osdol W W; Saga T; Neumann R D; Dedrick R L; Weinstein J N. (Biomedical Engineering and Instrumentation Program, National Center for Research Resources, NIH, Bethesda, Maryland 20892.)CANCER RESEARCH, (1994 Apr 15) 54 (8) 2166-75. Journal code: CNF. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB We have developed a pharmacokinetic model for the analysis of a protocol that involves injection of a biotinylated monoclonal antibody followed at a later time by radiolabeled **streptavidin**. Three distinct physiological spaces are described: an avascular tumor nodule, the normal tissue surrounding the tumor, and the plasma. The model incorporates processes such as plasma kinetics, transcapillary transport, interstitial diffusion, binding reactions, and lymphatic clearances. We have modeled cases in which antigen turnover does not occur, in which antigen turnover does occur (24-h time constant), and in which circulating antibody is cleared from the plasma immediately prior to injection of **streptavidin**. We have calculated the spatial and temporal distributions of a tumor-specific antibody and of **streptavidin** in the tumor nodule using parameter values that simulate conditions of recent experiments on metastatic nodules in the guinea pig lung. The

theoretical distribution of **streptavidin** in the tumor nodule shows an initial localization at the periphery that progresses to a fairly uniform distribution throughout the nodule, a temporal sequence that is very similar to experimental observation. This finding indicates that, in a tumor pretargeted with biotinylated antibody, **streptavidin** can encounter significant retardation in its penetration as a consequence of the high affinity interaction between these two species. Tumor:**blood** and tumor:lung ratios were calculated and compared to experimental results. In addition, the calculated tumor:**blood** ratios, tumor:lung ratios, and relative exposures were compared to values obtained from a model of one-step antibody delivery. The two-step protocol yielded an approximately 2- to 3-fold enhancement in these pharmacokinetic indices compared with the one-step method.

L83 ANSWER 19 OF 42 MEDLINE
94228537 Document Number: 94228537. Two-step targeting of experimental lung metastases with biotinylated antibody and radiolabeled **streptavidin**. Saga T; Weinstein J N; Jeong J M; Heya T; Lee J T; Le N; Paik C H; Sung C; Neumann R D. (Nuclear Medicine Department, Clinical Center, NIH, Bethesda, Maryland 20892.)CANCER RESEARCH, (1994 Apr 15) 54 (8) 2160-5. Journal code: CNF. ISSN: 0008-5472. Pub. country: United States. Language: English.
AB Two-step monoclonal antibody tumor targeting using an avidin-biotin system has unique characteristics because of the high-affinity binding (10(15) M-1) and the lower molecular weight ligands (avidin, **streptavidin**, or biotin) used as carriers of **radioisotopes**, toxins, or drugs. The distribution of radiolabeled **streptavidin** in a two-step targeting strategy was investigated in lung metastases of line 10 carcinoma in guinea pigs. The microdistribution of administered D3 monoclonal antibody and 125I-labeled **streptavidin** in metastatic nodules was examined by immunohistochemistry and autoradiography, and the uptake was quantitated. With monoclonal antibody pretargeting, **streptavidin** was found mainly at the periphery of metastatic nodules 1.5 h after injection; it had penetrated deeper at 4 h and was approaching homogeneity in many of the tumor nodules at 24 h. These results indicate that **streptavidin** can penetrate into metastatic nodules more rapidly than can the antibody. The concentration of **streptavidin** in metastatic nodules 4 h after injection was 5.6 times higher for the pretargeted group than for the nonpretargeted group, and the pretargeting index was 4.7. Although the absolute uptake of **streptavidin** had decreased between 4 and 24 h, the metastasis:**blood** ratio had increased from 1.2 to 2.4. When compared with the animals injected with 125I-labeled D3 antibody alone, the pretargeted group achieved higher tumor:**blood** and tumor:lung ratios and a higher localization index at early times after injection of the radiolabeled species.

L83 ANSWER 20 OF 42 MEDLINE
94322100 Document Number: 94322100. Immunotargeting of **streptavidin** to the pulmonary endothelium. Muzykantov V R; Atochina E N; Gavriljuk V; Danilov S M; Fisher A B. (Institute of Experimental Cardiology, Russian National Cardiology Research Center, Moscow.)JOURNAL OF NUCLEAR MEDICINE, (1994 Aug) 35 (8) 1358-65. Journal code: JEC. ISSN: 0161-5505. Pub. country: United States. Language: English.
AB We have observed previously that monoclonal antibody to

angiotensin-converting enzyme (Mab 9B9) accumulates selectively in the lung after intravenous injection. The objective of the present work is the development of a universal system for targeting of drug or radiolabel to the lung, using biotinylated Mab 9B9 and **streptavidin**. METHODS: Mab 9B9 was biotinylated with biotin succinimide ester (b-Mab 9B9), while **streptavidin** (SA) was radiolabeled with 125I. Interaction between b-Mab 9B9 and SA has been estimated in solid-phase radioassay. Radiolabeled SA was conjugated with b-Mab 9B9 or with b-IgG and injected intravenously in rats or perfused in isolated rat lungs. RESULTS: Radiolabeled b-Mab 9B9 biotinylated at biotin-to-antibody molar ratio 10 (b-Mab 9B9) retains its ability to accumulate in rat lungs after intravenous injection. Radiolabeled SA conjugated with b-Mab 9B9 accumulates in the lung tissue in perfused isolated rat lungs. About 20% of injected SA accumulates in the rat lung 1 hr after intravenous injection (localization ratio is 20, immunospecificity of the conjugate pulmonary uptake is 70). As compared with conjugate injection, stepwise intravenous injection of b-Mab 9B9 and radiolabeled SA leads to a marked reduction of SA pulmonary uptake. Maximal pulmonary uptake of Mab 9B9 has been observed 2-3 hr after intravenous injection, while 24 hr later, radioactivity in the lung was markedly reduced. In contrast to radiolabeled Mab 9B9 alone, radiolabeled SA conjugated with b-Mab 9B9 was retained in the lung for at least 48 hr. In concert with effective **blood** clearance of the conjugate, its prolonged lung retention leads to a marked increase in its lung-to-**blood** ratio: 80 for SA-b-Mab 9B9 versus 15-20 for Mab 9B9. CONCLUSION: Conjugation of Mab 9B9 with **streptavidin** enhances selective pulmonary uptake of the preparation, providing a background for intrapulmonary immunotargeting of various biotinylated agents.

L83 ANSWER 21 OF 42 MEDLINE

95079450 Document Number: 95079450. The fate of antibodies and their radiolabels bound to tumor cells in vitro: the effect of cross-linking at the cell surface and of anti-idiotype antibodies. Ong G L; Marria V; Mattes M J. (Garden State Cancer Center, Center for Molecular Medicine and Immunology, Newark, NJ 07103.)CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1994 Nov) 39 (5) 325-31. Journal code: CN3. ISSN: 0340-7004. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB In order to obtain rapid **blood** clearance of circulating antibodies (Ab) at a desired time, cross-linking reagents such as second Ab are often employed. Such reagents will generally bind to Ab located at the tumor site as well as free Ab, and we therefore investigated whether the cross-linking of Ab bound to the surface of tumor cells affects the processing of those Ab. Cross-linking was induced in various ways: a polyclonal second Ab [rabbit anti-(mouse IgG)], a monoclonal rat anti-(mouse IgG constant region) Ab, and **streptavidin** used in conjunction with a biotinylated first Ab. Processing was followed for 3 days, to allow nearly all of the bound Ab to reach its ultimate fate. Results depended strongly on the particular first Ab used. Two basic effects were observed. First, the second Ab efficiently prevented the early dissociation of intact Ab from the cell; once the second Ab bound, there was virtually no dissociation of the primary Ab bound to the cells. For most Ab, where only a small proportion of bound Ab dissociated intact, this effect was relatively small. However, for an unusual Ab, where the majority dissociated intact (L6) the effect of a second Ab in prolonging Ab retention by the cell was dramatic. Second, cross-linking sometimes resulted in markedly accelerated internalization and degradation of the bound Ab, coupled with the release of degradation products into the medium. This process resulted in much shorter retention of the

radioisotope by the cell. If a "residualizing" radiolabel was used, 125I-dilactitoltyramine, which is probably trapped within lysosomes after Ab catabolism, the effect of the second Ab in accelerating loss from the cell was largely prevented. We also tested anti-idiotype Ab as cross-linking reagents. In addition to testing anti-idiotype Ab known to react with the cell-bound primary Ab, we also tested anti-idiotype Ab not expected to bind to cell-bound Ab, initially as a negative control. Unexpectedly, all anti-idiotype Ab tested induced rapid release of the primary Ab from the cell. This effect was similar to the effect of a large excess of unlabeled Ab, and we attribute it to the blocking of the free binding site of a "wobbling" Ab, which prevents its rebinding to a second antigen molecule. We conclude that the use of selected anti-idiotype Ab to clear circulating Ab, while not reacting with cell-bound Ab, must be done cautiously.(ABSTRACT TRUNCATED AT 400 WORDS)

L83 ANSWER 22 OF 42 MEDLINE DUPLICATE 3
95123120 Document Number: 95123120. A sensitive ELISA for measuring the adhesion of leukocytic cells to human endothelial cells. Krakauer T. (Applied Research Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011..)JOURNAL OF IMMUNOLOGICAL METHODS, (1994 Dec 28) 177 (1-2) 207-13. Journal code: IFE. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.
AB A new, sensitive ELISA using monoclonal antibodies reactive with surface molecules specific for various leukocytes was devised to measure the attachment of these cells to cultured monolayers of human umbilical vein endothelial cells. Preparations of peripheral **blood** mononuclear cells, a human monocytic cell line (THP-1) and a human lymphoblastic T cell line (MOLT-4) were used to test the sensitivity of this method and compare it with the conventional 51Cr-radiolabeled cell assay. The extent of adhesion to endothelial cells was assayed by measuring the optical density produced by a complex of peroxidase-labeled **streptavidin**, biotin-conjugated F(ab')2 anti-mouse Ig and monoclonal antibody on fixed leukocytic cells that had adhered to endothelial cells. This method is fast and sensitive, eliminates the use of **radioisotopes**, and, because the detection uses a specific marker on the cell of interest, can be used in preparations of unseparated mixtures of cells. As this is a microassay, using relatively small number of cells and reagents, the methodology can be applied to screen a large number of therapeutic agents that may regulate adhesion. Using this method, the anti-inflammatory corticosteroid, dexamethasone, was found to inhibit the adhesion of THP-1 and MOLT-4 cells to **cytokine**-activated endothelial cells.

L83 ANSWER 23 OF 42 MEDLINE
95058324 Document Number: 95058324. Radioimmune western blotting in comparison with conventional western blotting, second and third generation ELISA assays for the serodiagnosis of HIV-1 infection. Portincasa P; Conti G; Zannino T; Visalli S; Chezzi C. (Istituto di Microbiologia, Facolt`a di Medicina e Chirurgia, Universit`a degli Studi di Parma, Italy.)NEW MICROBIOLOGICA, (1994 Jul) 17 (3) 169-76. Journal code: CGC. ISSN: 1121-7138. Pub. country: Italy. Language: English.
AB We compared the performance of second and third generation ELISA assays to detect antibodies to HIV-1 virus with conventional Western blotting (WB) and radioimmune Western blotting (RIWB). Both sera from commercial

seroconversion panels and serial dilutions of a serum for HIV-1 antibodies were tested with Murex HIV Recombinant, Vidas bioMerieux HIV 1/2 (2nd generation ELISA) Murex HIV 1-2 (3rd generation ELISA), as well as with WB and RIWB. In seroconversion panels all ELISA assays were positive for the same serum with the exception of the first serum of Panel D which was negative with both sample Murex assays and borderline with Vidas assay. This serum was negative with WB but evidenced antibodies to gp160 p66, p51, p24 HIV-1 **proteins** when assayed by RIWB. In only two cases did WB reveal antibodies to HIV-1 **proteins** before ELISA assays (Panel A and E); not only did RIWB show the same sensitivity as WB in the two last panels, but it also detected antibodies to HIV-1 **proteins** earlier than WB, ranging from a few days (Panel C) to approximately 12 weeks (Panel D). The results obtained by testing the dilutions of the serum positive for anti HIV-1 antibodies showed the following degrees of sensitivity: Murex HIV 1-2 (the most sensitive), Murex HIV Recombinant and Vidas bioMerieux HIV 1/2. Although WB was more sensitive than the ELISA assays and picked out antibodies to gp160, gp120 and p24 HIV **proteins** at 1/4000 serum dilution, the most sensitive test was RIWB which at 1/20,000 serum dilution enabled detection of antibodies to gp160, p66 and p24 HIV **proteins**.

L83 ANSWER 24 OF 42 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 4

1995:224722 Document No. 122:26366 Determination of GDP-Fuc:Gal.beta.1-4GlcNAc-R (Fuc to GlcNAc) .alpha.1,3 fucosyltransferase activity by a solid-phase method. Yan, Liying; Smith, David F.; Cummings, Richard D. (Department Biochemistry Molecular Biology, University Oklahoma Health Sciences Center, Oklahoma City, OK, 73190, USA). Anal. Biochem., 223(1), 111-18 (English) 1994. CODEN: ANBCA2. ISSN: 0003-2697.

AB We report the development of a solid-phase assay for the activity of the enzyme GDPFuc:Gal.beta.1-4GlcNAc-R (Fuc to GlcNAc) .alpha.1,3 fucosyltransferase (.alpha.1,3FT). This enzyme generates the **blood** group antigen Lewis x (Lex) Gal.beta.1-4(Fuc.alpha. 1-3)GlcNAc-R from the acceptor Gal.beta.1-4GlcNAc-R. In our method, the tetrasaccharide Gal.beta.1-4GlcNAc.beta.1-3Gal.beta.1-4Glc (lacto-N-neotetraose, LNnT) from human milk was chem. conjugated to bovine serum albumin (BSA) to generate LNnT-BSA. As a source of .alpha.1,3FT to develop the assay, we used exts. of COS7 cells created to stably express the human FucTIII and FucTIV genes, both of which have .alpha.1,3FT activity. LNnT-BSA was immobilized in microtiter wells and incubated with GDPFuc and cell exts. The Lex antigen generated by .alpha.1,3FT was detected with a monoclonal IgM antibody (anti-CD15). Binding of this IgM-type antibody to product was detected by one of two methods. Method 1 was based on the binding of alk. phosphatase-conjugated goat anti-mouse IgM. Method 2 was based on the binding of a **streptavidin** conjugate of the recombinant bioluminescent **protein** aequorin to biotinylated goat anti-mouse IgM. The .alpha.1,3FT assay was linear with respect to time (0-3 h), ext. added (0-40 .mu.g), and was dependent on GDPFuc (20 .mu.M optimal) and LNnT-BSA. Both methods 1 and 2 allowed measurement of .alpha.1,3FT in exts. of the human cell line HL-60. The activity of the .alpha.1,3FT in human serum was too low, however, to be detected with the colorimetric method 1, but the bioluminescent method 2 allowed detection of activity in as little as 5 .mu.L of serum. This new assay has many advantages over current methods employing **radioisotopes** and allows rapid measurement of the specific enzyme product.

L83 ANSWER 25 OF 42 MEDLINE
93364328 Document Number: 93364328. Pharmacokinetics and biodistribution of radiolabeled avidin, **streptavidin** and biotin. Rosebrough S F. (Department of Radiology, University of Rochester Medical Center, NY 14642.)NUCLEAR MEDICINE AND BIOLOGY, (1993 Jul) 20 (5) 663-8. Journal code: BOO. ISSN: 0969-8051. Pub. country: United States. Language: English.

AB The extraordinary high affinity of avidin and **streptavidin** for biotin may be exploited in a two-step approach for delivering radiolabeled biotin derivatives suitable for imaging and therapy to target-bound **streptavidin** or avidin conjugated monoclonal antibodies (MAbs). The in vivo pharmacokinetics and biodistribution of radiolabeled avidin, **streptavidin** (SA) and DTPA-biocytinamide (DTPA-biotin) were studied in the rabbit and dog. SA circulated in the **blood** similar to other 60 kDa **proteins**, avidin cleared immediately and DTPA-biotin exhibited plasma clearance by glomerular filtration.

L83 ANSWER 26 OF 42 MEDLINE
93192829 Document Number: 93192829. Improved tumor localization with (strept)avidin and labeled biotin as a substitute for antibody. Hnatowich D J; Fritz B; Virzi F; Mardirossian G; Rusckowski M. (Department of Nuclear Medicine, University of Massachusetts Medical Center, Worcester 01655.)NUCLEAR MEDICINE AND BIOLOGY, (1993 Feb) 20 (2) 189-95. Journal code: BOO. ISSN: 0969-8051. Pub. country: United States. Language: English.

AB Because of its short physical half life, the use of anti-tumor antibodies radiolabeled with 99mTc has necessitated early (i.e. 2-6 h post-administration) imaging. It is possible that at these early times localization of antibodies in certain tumors may be largely due to non-specific processes. If so, other **proteins** or agents may be preferred for early imaging of solid tumors. We have investigated tumor localization with labeled biotin administered subsequent to unlabeled and unconjugated **streptavidin**. Nude mice bearing anti-CEA tumors (LS174T) received 10 micrograms of 111In-labeled anti-CEA antibody (C110) or 111In-labeled **streptavidin** with sacrifice 5 h later. In an examination of pretargeting, other animals received 50 micrograms of unlabeled **streptavidin** followed 3 h later with 1 micrograms of 111In-labeled biotin (EB1) and sacrifice 2 h later. The biodistribution of labeled **streptavidin** was similar to that of labeled specific antibody except for lower **blood** and higher kidney levels. Tumor levels were also lower with labeled **streptavidin** but, because of still lower levels in liver and **blood**, the tumor/normal tissue ratios were improved. When unlabeled **streptavidin** was administered and followed by labeled biotin (pretargeting), tumor levels were further reduced modestly; however, normal tissue levels were greatly reduced such that the tumor/**blood** and tumor/liver ratios were 10.6 and 2.2 vs 1.5 and 0.5 for the specific antibody. Improvements were seen in all tissues sampled with the exception of kidney and muscle. A further control of labeled biotin alone (without the preinjection of **streptavidin**) showed minimal accumulations in all tissues with the exception of kidneys. (ABSTRACT TRUNCATED AT 250 WORDS)

L83 ANSWER 27 OF 42 MEDLINE
94057360 Document Number: 94057360. Detection of quantitative polymerase chain reaction products by hybridization on magnetic support with 125I-radiolabeled probes: quantification of c-myc copy numbers. Rhoer-Moja S; Cohen-Haguenauer O; Jouve C; Healy J C; Vindimian M. (Laboratoire de

Biophysique et d'Informatique Medical, UER de Medecine, Saint Etienne, France.)ANALYTICAL BIOCHEMISTRY, (1993 Aug 15) 213 (1) 12-8. Journal code: 4NK. ISSN: 0003-2697. Pub. country: United States. Language: English.

AB We present a technique for determining c-myc copy numbers that can be used as a prognosis index for some cancers. The method is based on the use of both competitive polymerase chain reaction and hybridization of amplified products. Coamplification was performed directly on cells with a synthetic oligonucleotide used as internal standard. It recognized the same primer set as the target. Coamplified products were captured on **streptavidin** magnetic beads as solid support using a 5' biotinylated primer. DNA immobilized on this support was denatured with alkali. Each coamplified product (target and reference gene) was further hybridized to two distinct specific oligonucleotide probes. Gene amplification levels were determined using a standard curve obtained by serial dilutions of peripheral **blood** lymphocytes run along with the experimental samples. This approach provides a rapid (less than 2 days) and reproducible method for evaluating c-myc gene copy number and may be used to quantify any gene. Moreover, its format allows for automation.

L83 ANSWER 28 OF 42 CAPLUS COPYRIGHT 1999 ACS

1992:620158 Document No. 117:220158 A system for enhanced in vivo clearance of diagnostic and/or therapeutic agents by extracorporeal depletion. Nilsson, Rune; Lindgren, Lars; Norrgren, Kristina; Sandberg, Bengt; Sjoegren, Hans Olof; Strand, Sven Erik (Swed.). PCT Int. Appl. WO 9212730 A1 19920806, 31 pp. DESIGNATED STATES: W: CA, FI, JP, NO, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 92-SE20 19920115. PRIORITY: SE 91-142 19910117.

AB Non-tissue-bound targeting mols., selective for certain tissues or cells, intended for diagnostic and/or therapeutic applications are removed from **blood** or reduced to a lower concn. by an extracorporeal system contg. immobilized agents (e.g. receptors) which selectively bind the targeting mols. Targeting mols. are antibodies or their fragments conjugated with cytotoxic drugs, **radioisotopes** or activators of prodrugs, i.e. enzymes.

L83 ANSWER 29 OF 42 MEDLINE

93019602 Document Number: 93019602. Localization of infection using **streptavidin** and biotin: an alternative to nonspecific polyclonal immunoglobulin [see comments]. Rusckowski M; Fritz B; Hnatowich D J. (Department of Nuclear Medicine, University of Massachusetts Medical Center, Worcester 01655.)JOURNAL OF NUCLEAR MEDICINE, (1992 Oct) 33 (10) 1810-5. Journal code: JEC. ISSN: 0161-5505. Pub. country: United States. Language: English.

AB Since favorable images of infection are obtained with radio-labeled nonspecific IgG, **streptavidin** has been considered as an alternative **protein** in this investigation. The advantage of **streptavidin** is that once localized it may be targeted with radiolabeled biotin. Studies were conducted in a mouse model with an Escherichia coli infection in one thigh. Indium-111-labeled **streptavidin** showed equivalent localization to the infection as that obtained with 111In-labeled polyclonal nonspecific IgG, however **blood** levels with **streptavidin** were lower at all time points; consequently, target-to-**blood** ratios were improved. Pretargeting with unlabeled **streptavidin** followed 3 hr later with 111In-labeled biotin showed equivalent localization in the target and

reduced activity in all organs sampled. As such, infected thigh-to-normal thigh ratios were improved 3-fold for pretargeting versus either labeled IgG or **streptavidin**. Improvements in infected thigh-to-liver and **blood** ratios were greater than 8-fold. Only in the case of kidneys was the ratio unimproved. In conclusion, we have shown that by preadministration of unlabeled **streptavidin** followed by labeled biotin, infectious lesions in a mouse model may be imaged earlier with lower background levels relative to the administration of labeled nonspecific IgG.

L83 ANSWER 30 OF 42 MEDLINE
92306977 Document Number: 92306977. Two-step tumour targetting in ovarian cancer patients using biotinylated monoclonal antibodies and radioactive **streptavidin**. Paganelli G; Belloni C; Magnani P; Zito F; Pasini A; Sassi I; Meroni M; Mariani M; Vignali M; Siccardi A G; et al. (Department of Nuclear Medicine, Istituto Scientifico H. San Raffaele, Sorin Biomedica, Universit`a di Milano, Italy.)EUROPEAN JOURNAL OF NUCLEAR MEDICINE, (1992) 19 (5) 322-9. Journal code: ENC. ISSN: 0340-6997. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB A new method for intraperitoneal tumour targetting in ovarian cancer using biotinylated monoclonal antibodies (MoAb) and radioactive **streptavidin** is described. Fifteen patients with histologically documented ovarian carcinoma were injected intraperitoneally with 2 mg of biotinylated MoAb MOv18, followed 3-5 days later by 100-150 micrograms of indium-111 **streptavidin**, at the specific activity of 280-370 MBq/mg in 500 ml of normal saline. No toxicity was observed. Tumours were imaged from 2 to 48 h after radioactivity injection by recording both planar and single photon emission tomography (SPET) data. All patients underwent surgery 1-8 days later (mean 3 days) after scanning. The resected tumour and normal tissue radioactivity were measured. On the day of surgery, the tumour to normal tissue ratio was 9:1 (range 3:1-30:1) and 45:1 (range 12:1-120:1) for intra- and extraperitoneal samples, respectively. The mean tumor to **blood** ratio was 14:1 (range 4:1-30:1). The injected dose (i.d.) per gram of tumour was 0.112 (range 0.01-0.3) for recurrences and 0.05 for primary tumour (range 0.005-0.2). Over 24-48 h 14% i.d. (range 8-18% i.d.) was found in the urine, 14% i.d. (range 6-29% i.d.) in the **blood** and 63% i.d. (range 56-70% i.d.) was still in the peritoneal cavity. These preliminary clinical data suggest that this two-step strategy may be superior to the conventional approach (radiolabelled antibodies) for intraperitoneal radioimmunolocalization and radioimmunotherapy of ovarian cancer.

L83 ANSWER 31 OF 42 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 91-171653 [24] WPIDS
CR 89-339974 [46]
AB CA 2026250 A UPAB: 950524
Purified p100 is claimed which is a human neu related **protein** having a mol. wt. of 97,000-115,000 daltons which corresponds to the extracellular domain of the human neu gene prod., the **protein** being detectable in a biological fluid.

Also claimed is a monoclonal antibody (MAb) which is capable of binding to p100 which is a human neu related **protein** having a mol. wt. of 97,000-115,000 daltons where the **protein** corresponds to the extracellular domain of the human neu gene prod., the **protein** being detectable in a biological fluid. The antibody may be conjugated to biotin and detected by reaction with **streptavidin** horseradish peroxidase and the o-phenylenediamine.

USE/ADVANTAGE - The MAbs can be used for detecting p100 in **body fluids** for detecting preneoplastic or neoplastic

cells in humans. @(70pp Dwg.No.0/18)
0/18

ABEQ US 5401638 A UPAB: 950518
Immunoassay for detecting the presence of p100 in a sample of human plasma
or serum comprises (a) reacting the sample with a 1st antibody which specifically binds an epitope of the extracellular domain of human neu gene encoded prod, (b) reacting the obtd prod with a detectably labelled 2nd antibody which specifically binds the extracellular domain of human neu gene encoded prod at an epitope different from the epitope bound by the 1st antibody, and (c) detecting the obtd prod to detect the presence of the p100.

Pref the p100 is a **protein** having a mol wt of 97-115 kD. The immunoreactive fragments of the antibodies are used. The detectable label comprises **radioisotopes**, enzymes, fluorogens, chemiluminescent cpds or electrochemicals. The 2nd antibody is conjugated to alkaline phosphatase. The 1st antibody is produced by hybridoma cell lines having ATCC accession numbers HB10205 or HB9689. The 2nd antibody is
similarly produced.
Dwg.1/18

ABEQ US 5604107 A UPAB: 970326
A novel immunoassay for detecting the overexpression of human neu gene encoded p185 **protein** in a sample of cells comprises: (a) obtaining a lysate of the sample of cells;

(b) contacting the lysate with an antibody specific for the extracellular domain of the human neu gene encoded p185 **protein** under conditions suitable for binding; and

(c) comparing the level of binding with the level of binding in a lysate of normal cells, wherein a higher level of binding is indicative of
the overexpression of the human neu gene encoded p185 **protein** in the sample of cells.
Dwg.0/18

L83 ANSWER 32 OF 42 MEDLINE

92155955 Document Number: 92155955. New indium-111 labeled biotin derivatives for improved immunotargeting. Virzi F; Fritz B; Rusckowski M; Gionet M; Misra H; Hnatowich D J. (Department of Nuclear Medicine, University of Massachusetts Medical Center, Worcester 01655.
)INTERNATIONAL JOURNAL OF RADIATION APPLICATIONS AND INSTRUMENTATION. PART
B, NUCLEAR MEDICINE AND BIOLOGY, (1991) 18 (7) 719-26. Journal code: G3J.
ISSN: 0883-2897. Pub. country: United States. Language: English.

AB Investigations into the use of **streptavidin**-conjugated antibodies and labeled biotin to improve radioimmunotargeting have shown background levels drastically reduced over the conventional approach. Nevertheless, accumulation of 111In-biotin in normal tissue as well as **streptavidin**-independent accumulation in tumor, was observed. In this work, the effect of altering the biotin molecule to reduce this nonspecific uptake without decreasing specific localization has been investigated. Three EDTA and DTPA derivatives of biotin have been synthesized and investigated along with a commercial biotin derivative (DTPA-B2). The labeled biotin chelates were administered i.p. to normal mice implanted with avidin beads in one thigh. A wide variation in biodistribution was seen among the biotin derivatives. The most favorable results were obtained with biotinyl-hydrazino-EDTA (EDTA-B1), which showed
the lowest accumulation in normal tissues but equivalent uptake in the target with respect to the other compounds. Averaged over 8 tissues

sampled, the target-to-nontarget ratio was 140 vs 9 for EDTA-B1 vs DTPA-B2
(N = 6) at 24 h post administration. Similar observations have been made in culture with two tumor cell lines: positive accumulation of both DTPA-B2 and EDTA-B1 was measured in tumor cells independent of **streptavidin**-antibody conjugate, however in the case of the latter derivative, this accumulation was 3-5 fold lower. These studies show that modification of the biotin species can alter accumulation in normal tissues as well as the antibody-**streptavidin** independent accumulation in tumor tissue.

L83 ANSWER 33 OF 42 MEDLINE
91038321 Document Number: 91038321. Imaging of tumor in patients with indium-111-labeled biotin and **streptavidin**-conjugated antibodies: preliminary communication [see comments]. Kalofonos H P; Rusckowski M; Siebecker D A; Sivolapenko G B; Snook D; Lavender J P; Epenetos A A; Hnatowich D J. (Royal Postgraduate Medical School, Hammersmith Hospital, London, United Kingdom.)JOURNAL OF NUCLEAR MEDICINE, (1990 Nov) 31 (11) 1791-6. Journal code: JEC. ISSN: 0161-5505. Pub. country: United States. Language: English.
AB Tumor localization in patients has been achieved through the in vivo use of **streptavidin** and biotin. In these preliminary studies, the monoclonal antibody HMFG1 was conjugated with **streptavidin** and 1 mg was administered intravenously to each of 10 patients with documented squamous cell carcinoma of the lung. Two to 3 days later, 111In-labeled biotin was also administered intravenously. No evidence of toxicity was observed. Background radioactivity levels were reduced in liver (1% ID at 24 hr) and kidneys (2%) and in all other normal tissues and **blood** . Images of lung tumor were obtained in as little as 2 hr following administration of labeled biotin. In eight patients, tumor was detected with labeled biotin alone without the previous administration of **streptavidin**-conjugated antibody but in three of these patients, the images were improved with the prior administration of conjugated antibody. These results suggest that this approach may improve the tumor-to-normal tissue radioactivity ratios in radioimmunotargeting.

L83 ANSWER 34 OF 42 MEDLINE
90277260 Document Number: 90277260. Intraperitoneal radio-localization of tumors pre-targeted by biotinylated monoclonal antibodies. Paganelli G; Pervez S; Siccardi A G; Rowlinson G; Deleide G; Chiolerio F; Malcovati M; Scassellati G A; Epenetos A A. (Ospedale M. Bufalini, Cesena, Italy.)INTERNATIONAL JOURNAL OF CANCER, (1990 Jun 15) 45 (6) 1184-9. Journal code: GQU. ISSN: 0020-7136. Pub. country: United States. Language: English.
AB We describe 2-step and 3-step strategies for intraperitoneal tumor radio-localization by means of monoclonal antibodies (MAbs). Nude mice bearing intraperitoneal human colon carcinoma tumors were injected i.p. with biotinylated MAb AUA1, followed 24 hr later by radioiodinated **streptavidin** (2-step). The uptake of radioactivity in tumor and normal tissues was measured 4 hr after injection of radioactive compound. A 3-step strategy consisted in administering biotinylated antibody, cold avidin after 24 hr and 111In-labelled biotin after a further 4 hr; mice were then killed 2 hr later. Tumor localization of intraperitoneally-administered biotinylated antibody and direct targeting of radioactive **streptavidin** to biotinylated antibody bound to tumor sites were demonstrated using immunohistochemistry and autoradiography. Our results show that (i) the 2-step approach increased the percentage of radioactivity uptake by tumor with respect to directly labelled antibodies
(24% vs. 6%) and improved the tumor/non-tumor ratio; (ii) the 3-step approach allowed faster **blood** clearance of the radioactive probe

(111In-biotin) and yielded high tumor/non-tumor ratios. "Pre-targeting" methods appear to have advantages over the conventional 1-step approach with directly radiolabelled antibody.

L83 ANSWER 35 OF 42 MEDLINE DUPLICATE 5
90124270 Document Number: 90124270. Disulfide bond-targeted radiolabeling: tumor specificity of a **streptavidin**-biotinylated monoclonal antibody complex. del Rosario R B; Wahl R L. (Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor 48109-0028.)CANCER RESEARCH, (1990 Feb 1) 50 (3 Suppl) 804s-808s. Journal code: CNF.
ISSN: 0008-5472. Pub. country: United States. Language: English.
AB A site-specific labeling method was developed in which sulfhydryl groups of a murine IgG2a anti-ovarian monoclonal antibody, 5G6.4, were biotinylated with N-iodoacetyl-N'-biotinylhexylenediamine (Compound 1) following partial reduction of disulfide bonds with dithiothreitol. Reaction of 1-alkylated 5G6.4 with 125I-**streptavidin** gave immunoreactive **streptavidin**-1-biotinylated complexes. Radio-fast **protein** liquid chromatography data were consistent with the formation of a stable monovalent **streptavidin**-half-antibody complex as the major species. In vivo specific localization of these radioantibody conjugates to human tumor xenografts of ovarian carcinoma was confirmed by a comparative biodistribution study in nude mice using as a control the nonspecific 125I-**streptavidin**-1-alkylated UPC-10 (an irrelevant IgG2a monoclonal antibody) complex prepared analogously as described above. Tumor uptake for radiolabeled 5G6.4 [0.279 +/- 0.041% (SE) kg injection dose/g) was significantly greater [P less than 0.025] than for UPC-10 [0.165 +/- 0.027% kg injection dose/g]. The tumor: **blood** ratio (7.38 +/- 1.285) for 5G6.4 was approximately 3 times that for UPC-10 (2.48 +/- 0.708, P less than 0.01). This sulfhydryl site-directed approach demonstrated that reduced disulfides of monoclonal antibodies are viable sites for attaching labels without significant loss of in vitro and in vivo immunoreactivity.

L83 ANSWER 36 OF 42 MEDLINE
90249376 Document Number: 90249376. Tissue distribution of avidin and **streptavidin** injected to mice. Effect of avidin carbohydrate, **streptavidin** truncation and exogenous biotin. Schechter B; Silberman R; Arnon R; Wilchek M. (Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.)EUROPEAN JOURNAL OF BIOCHEMISTRY, (1990 Apr 30) 189 (2) 327-31. Journal code: EMZ. ISSN: 0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB Radioionated avidin and **streptavidin** were characterized for their biodistribution and tissue association in Balb/c mice, in comparison to their interaction with cells in vitro. Binding of avidin to spleen and bone-marrow cells in vitro was up to 20-fold higher than that of **streptavidin**, but when tested in vivo avidin clearance from **blood** and tissues was considerably faster than that of **streptavidin**. Levels of avidin at 24 h after an intravenous injection were below 1% (of the injected dose/mass tissue) in most organs. Non-glycosylated avidin was similar in its biodistribution to native avidin. Native **streptavidin** exhibited higher and prolonged tissue association with 5-10% levels in lung, liver, spleen, kidney and **blood**, whereas its truncated form showed low tissue levels (1-3%) but a remarkably high affinity to the kidney (80%). Exogenous biotin did not affect **streptavidin** distribution in vivo but caused a 2-7-fold increase in the retention of avidin (but not non-glycodylated

avidin) in some of the organs.

L83 ANSWER 37 OF 42 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
89148451 EMBASE Document No.: 1989148451. A simple time-resolved fluoroimmunoassay of total thyroxine in serum. Papanastasiou-Diamandis A.;
Bhayana V.; Diamandis E.P.. Department of Clinical Biochemistry, University of Toronto, Toronto, Ont. M5G 1L5, Canada. Annals of Clinical Biochemistry 26/3 (238-243) 1989.
ISSN: 0004-5632. CODEN: ACBOBU. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB We describe a non-isotopic heterogeneous competitive immunoassay of total thyroxine in serum. Thyroxine, released from its binding **proteins** by merthiolate (thimerosal), competes with immobilised thyroxine (thyroxine-bovine globulin conjugate) for binding to a monoclonal biotinylated antibody. The amount of biotinylated antibody bound, which is inversely related to the amount of thyroxine in the sample, is then quantified by adding **streptavidin** labelled with the europium chelator 4,7-bis(chlorosulphophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid (BCPDA) in the presence of excess Eu3+. The complex formed (bovine globulin-thyroxine-antibody-biotin-**streptavidin**-BCPDA-Eu3+) is measured on the solid-phase by time-resolved fluorescence. The assay is simple to perform and its characteristics are similar to those of other currently used immunoassay techniques.

L83 ANSWER 38 OF 42 MEDLINE
88223767 Document Number: 88223767. Colorimetric assay of **blood** coagulation factor XIII in plasma. Lee K N; Birckbichler P J; Patterson M K Jr. (Samuel Roberts Noble Foundation, Inc., Biomedical Division, Ardmore, OK 73402..)CLINICAL CHEMISTRY, (1988 May) 34 (5) 906-10.
Journal code: DBZ. ISSN: 0009-9147. Pub. country: United States. Language:
English.
AB In this new colorimetric assay for Factor XIII in plasma, 5-(biotinamido)pentylamine is used as the amine substrate. Factor XIII, a zymogen, is transformed by thrombin and Ca2+ to active Factor XIIIa, and the incorporation of 5-(biotinamido)pentylamine into N,N-dimethylcasein is used to measure catalytically active Factor XIIIa. The biotinylated enzymatic product is immobilized onto 96-well microtiter plates, complexed with **streptavidin**-beta-galactosidase, and the absorbance at 405 nm is monitored for production of p-nitrophenol from p-nitrophenyl-beta-D-galactopyranoside. Concentrations of N,N-dimethylcasein, 5-(biotinamido)pentylamine, Ca2+, and thrombin were chosen to allow near-maximum velocity of amine incorporation. A linear relationship was obtained between assay product and plasma volume, from 0.5 to 50 microL of plasma. Results correlated well (r greater than 0.924) with those from the most frequently utilized radiometric filter-paper assay for Factor XIII. The method appears to be ideal for routine diagnostic estimation of Factor XIII in plasma because of its simplicity, its lack of use of **radioisotopes**, and its potential for assay of large numbers of samples by use of microtiter plates and automated plate readers.

L83 ANSWER 39 OF 42 MEDLINE DUPLICATE 6
88187114 Document Number: 88187114. IgM/IgG solid-phase antibody-capture

assay with biotin/125I-**streptavidin** amplification: application to normal human sural nerve biopsies. Poduslo J F; Curran G L; Brunden K R; Dyck P J. (Department of Neurology, Mayo Clinic, Rochester, MN 55905.)JOURNAL OF NEUROIMMUNOLOGY, (1988 May) 18 (2) 117-24. Journal code: HSO.

ISSN: 0165-5728. Pub. country: Netherlands. Language: English.

AB A highly sensitive and specific solid-phase antibody-capture assay was developed to measure IgM and IgG in endoneurial preparations of human sural nerve biopsies. Assay amplification was obtained by utilizing biotin-labeled anti-IgM or anti-IgG antibody and 125I-**streptavidin**, resulting in multiple **streptavidin** molecules binding per biotinylated antibody molecule. A minimal detectable dose of 0.16 +/- 0.08 ng (mean +/- SD; n = 7) for IgM and 0.03 +/- 0.02 ng (mean +/- SD; n = 5) for IgG was obtained in a 100 microliters sample. When this assay was applied to normal fascicular biopsies from human sural nerve, the percent of IgM and IgG, respectively, of the total endoneurial **protein** was 0.026 +/- 0.015% (n = 9) and 0.27 +/- 0.15% (n = 10; mean +/- SD). When these endoneurial concentrations of IgM and IgG were related to the plasma concentrations (mg IgM or IgG/mg total plasma **protein**), an IgM-**blood**-nerve barrier (BNB) index of 4.09 +/- 1.95 and an IgG-BNB index of 2.07 +/- 1.10 were obtained (X10(2); mean +/- SD). These values were also related to the albumin (Alb) concentration in the biopsies as a percent of total endoneurial **protein** (2.48 +/- 1.07%; mean +/- SD) and with the Alb-BNB index (5.40 +/- 2.53; X10(2); mean +/- SD; n = 10). Although these normal values will be expected to change with age, sex, nerve, and proximal-distal distance from nerve root, they should provide a basis for the comparison of BNB indices from patients with peripheral neuropathy.

L83 ANSWER 40 OF 42 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.

87090157 EMBASE Document No.: 1987090157. Comparison of monoclonal and polyconal antibodies to cotinine in nonisotopic and isotopic immunoassays.

Bjercke R.J.; Cook G.; Langone J.J.. Department of Medicine, Baylor College of Medicine, Houston, TX 77030, United States. Journal of Immunological Methods 96/2 (239-246) 1987.

CODEN: JIMMBG. Pub. Country: Netherlands. Language: English.

AB Monoclonal antibodies (McAb) were used to develop nonisotopic and radioimmunoassays (RIA) for quantitative determination of the major nicotine metabolite, cotinine, in physiological fluids. ELISAs and fluorescence immunoassays were carried out in microtiter plate wells coated with a conjugate of cotinine 4'-carboxylic acid bound covalently to poly-L-lysine. The detection systems were horseradish peroxidase (HRP)-labeled staphylococcal **protein** A, HRP-**streptavidin**-biotin, and biotinylated alkaline phosphatase-4-methylumbelliferyl phosphate. With the three McAb tested, I50 values ranged between 0.024-0.063 ng cotinine and as little as 0.005-0.015 ng gave 15% inhibition. These assays were 5-20 times more sensitive than similar assays using six rabbit antisera. With McAb the standard inhibition curves

eeper and complete inhibition of immune binding was achieved with mately 1 ng cotinine. In contrast, 100-500 ng cotinine failed to eater than 80-90% inhibition with rabbit antibodies either in the ssays or in RIA using a 125I-labeled tyramine derivative of e as the tracer. In this RIA, the sensitivity with McAb (mean I50 ng cotinine) was over three-fold greater than with rabbit

0 of 1.84 ng). The presence of antibodies directed to the amide

linkage group common to the polylysine conjugate, 125I-tyramine derivative and the immunogen likely accounts for the inferior quality of assays using rabbit antisera. Consistent with this conclusion, superimposable inhibition curves were obtained in the RIA when monoclonal or rabbit antibodies were used with [3H]cotinine. Cotinine levels in **saliva**, serum and plasma from smokers and non-smokers determined with McAb-based assays showed a strong correlation with values obtained by RIA using rabbit antisera or by gas chromatography. Properly selected McAb offer distinct advantages over conventional antisera in nonisotopic immunoassays and RIAs for cotinine as a biochemical marker of active or passive smoking.

L83 ANSWER 41 OF 42 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
86047572 EMBASE Document No.: 1986047572. Drug-**protein** conjugates. IX. Immunogenicity of captopril-**protein** conjugates. Yeung J.H.K.; Coleman J.W.; Park B.K.. Department of Pharmacology & Therapeutics, University of Liverpool, Liverpool L69 3BX, United Kingdom. Biochemical Pharmacology 34/22 (4005-4012) 1985.
CODEN: BCPCA6. Pub. Country: United Kingdom. Language: English.
AB The immunogenicity of captopril (CP), conjugated to heterologous **proteins**, was investigated in male New Zealand White rabbits by monthly injections of CP-**protein** conjugates in Freund's Complete Adjuvant. Anti-CP antibody activity was readily detected by immunodiffusion in sera of rabbits immunized with the amide-linked CP-HSA (23:1) conjugate. Hapten inhibition studies revealed that the antigenic determinant contained CP and a lysine residue from the **protein** carrier. When rabbits were immunized with disulphide-linked CP-S-S-HSA (9:1) and CP-S-S-KLH (160:1) conjugates, anti-CP antibody activity was detected by a sensitive ELISA method, but not by immunodiffusion and radioligand binding assays. The specificity of the serum IgG anti-CP activity after immunization with disulphide-linked CP-S-S-**protein** conjugates was confirmed since anti-CP activity was inhibited by preincubation of the antisera with CP conjugated to an unrelated **protein** carrier (CP-S-S-OVA), but not by the corresponding unconjugated **protein**, nor by penicillamine-S-S-**protein** conjugates. These results show that disulphide-linked CP-**protein** conjugates are sufficiently stable to induce humoral (B lymphocyte) **anti-hapten** responses under the experimental conditions employed. In a separate study, delayed-type skin hypersensitivity reactions to topically applied CP were demonstrated in the guinea pig. The specific and sensitive immunochemical technique (ELISA) described here could be useful in future studies for determining whether or not patients taking CP produce antibodies to the drug.

L83 ANSWER 42 OF 42 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
85247296 EMBASE Document No.: 1985247296. The role of metabolism in the immunogenicity of drugs: Production of antibodies to a horseradish peroxidase generated conjugate paracetamol. Chesham J.; Davis G.E.. Immunology Group, Biomedical Sciences Section, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Nr Macclesfield, Cheshire SK10 4TJ, United Kingdom. Clinical and Experimental Immunology 61/2 (224-231) 1985.
CODEN: CEXIAL. Pub. Country: United Kingdom. Language: English.
AB The allergic response to small chemically inert molecules in thought to require their enzymatic conversion to reactive metabolites which are then endowed with the capacity to bind covalently to host **proteins**